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We take this opportunity to express our gratitude to the Maharaja Sayajirao University of Baroda for a very generous donation given towards the cost of the printing and publication of this journal for the year 1957-58.

CONTENTS

ON THE FLIGHT MUSCLES AND THE ASSOCIATED SKELETAL PARTS IN *STERNOCERA CHRYSIS* VAR. *CHRYSIDOIDES* (Fam. *BUPRESTIDAE*, Order *COLEOPTERA*)

K. R. Menon* and N. K. Joshi

SNODGRASS (1909) showed a fundamental similarity in the flight musculature and the associated sclerites in the thorax of insects. However, serious attempts to study the morphological and physiological details as well as the aerodynamic aspects of the flight mechanism have been made only recently. Notable among these is the work of Tiegs (1955) on Thysanura, Orthoptera, Hemiptera and Diptera. Jackson (1956) made interesting observations on flying and flightless water beetles. Chadwick (1953) has reviewed the whole subject with special reference to the relations of the leg and flight muscles of insects. It should be pointed out that little work has been done along these lines on Coleoptera, a group consisting of insects which, though less efficient in long range sustained flights, possess a number of interesting characteristics. It is a homogenous group in which exists the maximum heterogeneity as regards habits and habitats, and comprises of non-fliers as well as good fliers among the terrestrial and aquatic forms. Therefore, a study of the morphological peculiarities of the skeleto-muscular system of beetles should, it is hoped, throw some light not only on the changes effected in them as a result of their varied adaptations but also on the mechanism of flight. For the present investigation, the Buprestid beetle *Sternocera chrysis* Var. *chrysidoides* which is a good flying form and is believed to present a generalized flight musculature, has been selected for the study of its flight muscles and their associated skeletal parts.

Material and Method

The beetles were collected during the months of October, November and December, from Acacia shrubs of the Poona University campus. They were preserved in either 70% alcohol or alcoholic Bouins fluid. Skeletal preparations were made in the usual manner by boiling in 10% KOH.

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Observations

Sternocera chrysis Var. *chrysidoides* is capable of long flights and generally flies about during the sunny hours of the day. After flying for some time they come to rest on trees, usually clinging to branches by means of their legs with head directed downwards. They are not quite comfortable on the ground and do not take to walking.

External Features

Average length of the body ranges from 4 to 6 cm. and breadth of the thorax is about 2 cm. The whole body is built on a very sturdy plan and presents a bright copper colour on the dorsal side mainly due to the coloured elytra. The prothorax and head are black, the former presenting a pitted surface. Head is generally retracted a bit and hypognathus.

The thorax (Fig. 1) presents all the peculiarities characteristic of insects which are good fliers, but with comparatively poor terrestrial locomotion. Whole of the mesothorax, metathorax and abdominal tergites are covered by the elytra when the animal is at rest. On the ventral side, mesothorax and metathorax bear forwardly directed spines and all the thoracic sterna are interlocked firmly.

Legs are very poorly developed and are generally kept folded close to the thorax. Coxae are of two types. The prothoracic and mesothoracic legs have globose coxae and have monocondylic pleural attachment, the coxae being free to move in a coxal cavity in an aterio-posterior axis. Legs can be moved slightly at right angles to this axis due to the coxo-trochanteral articulations being made at right angles to the coxopleural joint. Individual segments of the leg can be moved freely and claws are especially useful for clinging purpose.

Metathoracic legs, though very similar to the first and second, have different coxal articulation. Coxa, instead of being globose and free to move in a ball and socket manner, is rigidly fixed to the sternum throughout its length and breadth. It is also quite large and flattened and provides a very big articular rim internally to make room for a heavy musculature for flight.

Prothorax:

Prothorax (Fig. 2) is the most heavily built and sturdy part of the thorax, and is quite big as compared to mesothorax. The tergum is a

single stout piece, and covers the dorsal surface and also extends well on to the lateral sides. Anteriorly the tergal plate is somewhat narrower but is broader posteriorly. Pronotum has no internal infolding of the body wall anteriorly but has a thick ridge, the antecosta on the posterior side (Fig. 2 b). On the ventral side, legs are attached by a ball and socket mechanism to the coxae and both the pleuron and the sternum are highly reduced. Positionally the legs are ventral and the coxae are free to rotate. Contrary to general observation in Coleoptera the prothorax and mesothorax do not resemble each other in morphological details.

Propleuron is highly reduced and the usual sclerites *i.e.* episternum and epimeron cannot be distinguished. However, a small sclerite—Trochantin—can be seen apposed to a process of the globose coxa, and these together form a ball and socket mechanism. Internally trochantin gives a process in the prothorax (Fig. 2 b) which is apposed to the lateral wall of the pronotum. The process is a rodlike extension of the trochantin and flattens at the end as shown in the figure. It provides surface area for the attachment of two muscles which control the movement of the coxa.

Prosternum is a small T-shaped sclerite which surrounds the coxa on the anterior side and also separates the two coxae forming a tongue-like piece in the centre. Arising from the centre of the sternum is a process which projects into the body cavity—the sternal apophysis (Fig. 2 b) or the furcasternum. The furcasternum has a body and two weakly developed arms, which are closely applied to the coxa internally and form a part of the socket into which the coxae fit. Prosternum which usually extends into the mesothorax fits into a concavity of the sternum of the mesothorax.

Mesothorax :

Mesothorax (Fig. 1 and 3) is reduced in size, especially in the notal region, while the pleural wall is quite well developed. Mesonotum (Fig. 3) consists of the Scutum which is formed of two plates which are roughly rectangular in shape and are closely apposed to each other mesially. Scutellum is a singly triangular piece. There is no pseudonotum but two membranous sclerites, referred to by Snodgrass (1909) can be seen.

Mesopleuron (Fig. 4) is quite well developed and the pleural suture is oblique. Unlike that of prothorax, mesothoracic pleuron can be divided into episternum and epimeron, with pleural suture visible externally. A

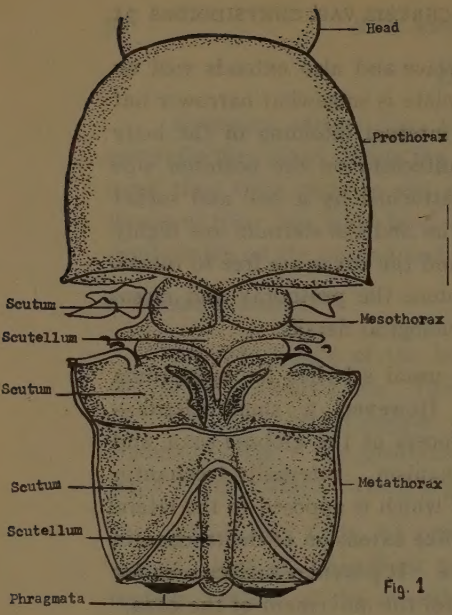
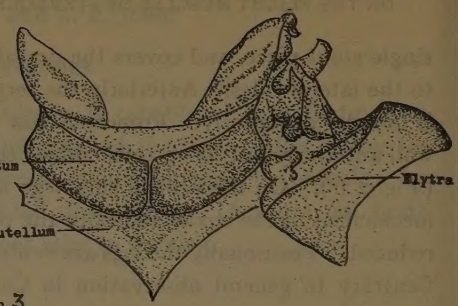


Fig. 1



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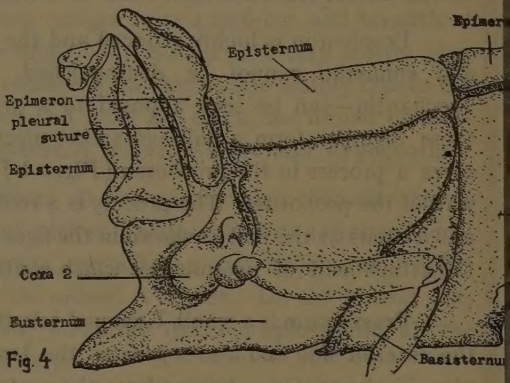

Fig. 4

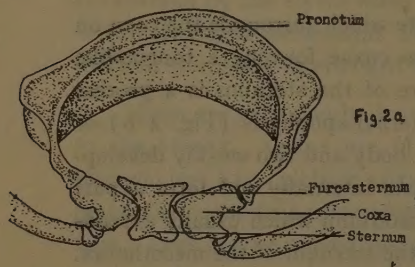

Fig. 2a

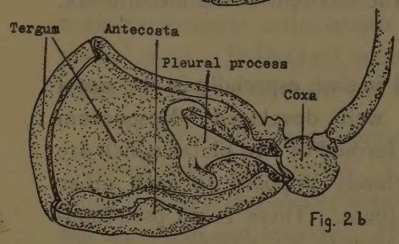

Fig. 2 b

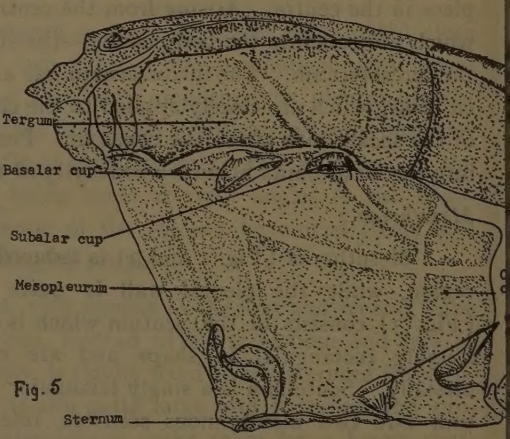

Fig. 5

Fig. 1. Dorsal view of thorax. Elytra and wings cut.
Fig. 2a. Prothorax viewed from behind showing furcasternum.
Fig. 2b. Prothorax cut in median plane to show pleural process.
Fig. 3. Dorsal view of mesotergum and base of the right elyron.
Fig. 4. External view of pleural wall of the mesothorax and metathorax.
Fig. 5. Metathorax showing endoskeleton. A part of the mesothorax also shown.

pleural ridge is present internally. Trochantin is present, but is hidden under the pleurites and is small as compared to that in prothorax.

Mesosternum is entire and is very similar to that of prosternum, except that it is thick and short. It also, like prosternum contributes towards the formation of the coxal socket. In shape the mesosternum resembles the prosternum but has a depression into which the metathoracic spine fits.

Metahorax :

Metathorax (Fig. 1) is a more complex structure and all the modifications are in order to accomodate and give proper attachment to the flight muscles.

Metanotum (Fig. 1) is distinctly divided into three transverse parts- prescutum, scutum and scutellum. The prescutum carries the prephragma and the anterior notal wing processes. The scutum is divided transversely into an anterior and a posterior plate by a large transverse ridge, and the surface shows a number of lines. These are again separated along the middle by a median interlocking of the prescutum and scutellum. Thus the scutum consists of four well marked subdivisions, the posterior pair of which carries the posterior notal wing processes. The scutellum is not very well marked. Apodemal cups for the insertion of tergo-coxal muscles are seen to arise from the anteriormost region of the scutum on its inner aspect (Fig. 5).

Wing attachment is as usual with three axillary sclerites. Metathoracic pleuron (Fig. 4) consists of two rectangular plates, episternum lying above the eusternum, while epimeron, a comparatively shorter selerite in length than the episternum, lies exactly above the fused coxa. On the inner side, the basalar and subalar apodemal cups are seen (Fig. 5) giving insertion to the subalar and basalar muscles.

Sternum (Fig. 4) consists of two distinct sclerites of unequal size. The one which is smaller in breadth is apposed to the coxa along its entire length and consists of a complicated structure inside, the sternal apophysis or the furcasternum. A pair of feeble arms of the furcasternum runs transversely and gets attached to the coxal wall. On the inner aspect there is no pleural ridge but episternum and eusternum are separated by a very thick ridge. Supraepimeral sclerites are absent.

The metathoracic coxa is a highly specialized structure, which has become completely fused with the body wall. Inside it is continuous with the metathoracic cavity and presents a suitable surface for the attachment of flight muscles. Just above the coxo-trochanteral joint and in the coxal cavity itself is to be found a large apodemal cup to which is attached a very big tergo-coxal muscle (Fig. 5). Again at the junction of the coxal wall with the sternum, just below the furcasternum is found a small apodemal cup for the attachment of another tergo-coxal muscle.

Pseudonotum is very well developed and carries the post-phragma and articulates with the epimeron on its extremity.

Myology

That the first two segments of the thorax, namely the pro-and mesothorax are not concerned in flight is clear from the poor development of their musculature. It is only the metathorax which is provided with an elaborate musculature that is concerned with flight. But for comparison, important muscles especially those concerned with the motion of the legs are described even in the pro-and mesothoracic segments.

Prothorax :

There are only three chief muscles developed in relation to the movement of the globose coxa and also the movement of the leg as a whole. Individual muscles of the leg segments are not described.

Tergo-coxal muscle (Fig. 6): This muscle originates in the anteriormost region of the tergum, slightly to the lateral side as a bundle of thick opaque fibres, but runs obliquely to the posterior side and ultimately is inserted on the posterior lateral rim of the coxa. The muscle is conical in shape and tapers down at about midway into a fine transparent tendinous portion. It is a tergal remoter of the coxa.

Pleuro-coxal muscle (Fig. 6): This muscle takes origin on the process of the trochantin, which projects into the prothorax. It arises on the expanded anterior half of the process as a thick bundle of short fibres and runs vertically downwards forming a tendinous stout portion which is inserted on the anterior rim of the coxa. The muscle by its contraction moves the coxa anteriorly and works antagonistic to the tergo-coxal described above.

Pleuro-trochanteral muscle (Fig. 6): This muscle also arises on the process of the trochantin and lies on the posterior side of the pleuro-coxal

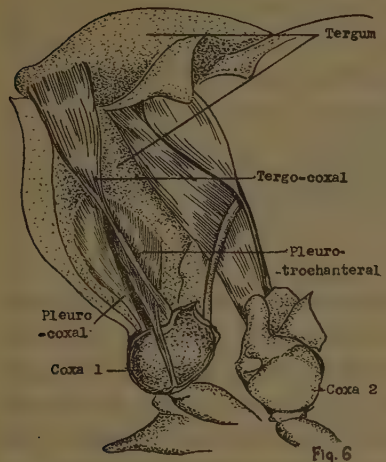

Fig. 6

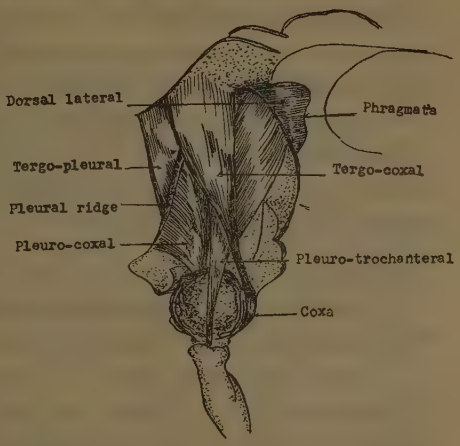

Fig. 7

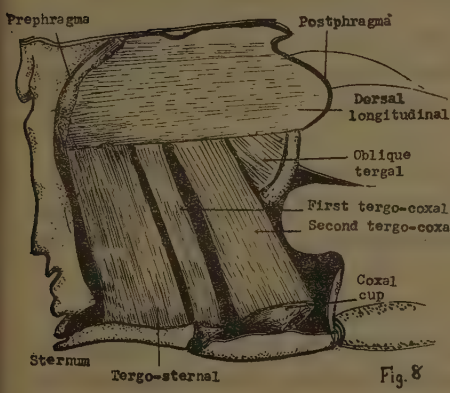

Fig. 8

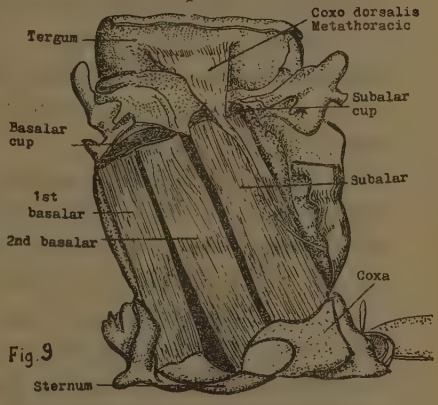

Fig. 9

Fig. 6. Prothorax-median dissection showing leg muscles. A part of the mesothorax with some muscles of the second leg also shown.
Fig. 7. Median dissection of mesothorax showing leg and wing muscles.
Fig. 8. Median dissection of metathorax showing flight and coxal muscles.
Fig. 9. Median dissection of metathorax after removing the muscles shown in Fig. 8.

muscle. This muscle also takes origin as a bundle of thick fibres, but tapers down to a fine tendon near the coxa and instead of being inserted on it, runs inside the coxa and is inserted on the trochanter at the coxo-trochanteral joint. This muscle moves the leg from trochanter downwards.

Dorsal muscles: In addition to the leg muscles described above, large number of muscle fibres run dorsally from the anterior end of the prothorax to the posterior where they are inserted on the mesothoracic tergum which is intimately connected with the prothorax.

Muscle fibres of short length take origin on the furcasternum and are attached to the outer lateral margin of the coxa. They do not appear to have any function in coxal movement. Both the dorsal and the furcal muscles are not shown in Fig. 6.

Mesothorax:

Leg muscles of the mesothorax are very similar to those of the prothorax, the mode of action of the two legs being very similar.

Pleuro-coxal muscle (Fig. 7): This muscle takes origin on the pleural ridge as a broad sheet of fibres, runs slightly obliquely to the posterior side and is inserted on the anterior rim of the coxa. Forward movement of the coxa is brought about by the contraction of this muscle.

Tergo-coxal muscle (Fig. 7): This muscle originates on the anterior side of the tergum and is inserted on the postero-lateral rim of the coxa. It has an antagonistic action to the pleuro-coxal muscle and is a tergal remotor.

Pleuro-trochanteral muscle (Fig. 7): This muscle takes origin on the posterior side, on the epimeron, and runs slightly down on to the anterior side, tepering into a fine tendon. It is inserted on the trochanter at the coxo-trochanteral joint.

Dorsal muscle (Fig. 7): This really constitutes the indirect wing levator muscle, but as the elytra are held passively during flight, these are not well developed. There is therefore no antagonistic dorso-ventral tergo-sternal muscle present in the mesothorax. Its fibres are very short and are inserted on the second phragma.

Tergo-pleural muscle (Fig. 7): This muscle originates on the anterior most region of the tergum and is inserted on the pleural ridge. It has no importance either in flight or leg movement.

Metathorax:

Though important muscles in the metathorax can be grouped into two as flight and leg muscles, evidence suggests that even the leg muscles especially the dorso-ventral tergo-coxal muscles contribute towards the deformation of the thoracic wall.

It has been customary to distinguish between two types of mechanisms in moving the wings, the direct and indirect, and the muscles of may thus be grouped accordingly.

The skeletal structures concerned in flight are the tergum and the pleura, with their endoskeletal extensions. Sternum or its derivatives play but a very little part. The indirect mechanism of wing movement consists of the wing articulations of the tergal wall situated mesad of the pleural fulcrum and the wing will be moved upon the pleural fulcrum by deformations of the thoracic cage which shifts the relative positions of the bearing surfaces. The direct mechanism consists of the wing muscles inserted on or near the wing base. The muscles which effect the movements of the wings may therefore be grouped into indirect and direct wing muscles.

Indirect muscles:

There are two sets of these muscles, one consisting of the dorsal lateral muscles and the oblique lateral muscles situated on the tergal wall and running antero-posteriorly and the other consisting of dorso-ventral or tergo-sternal muscles. By alternate contractions of these muscles the wing is moved up and down on a pleural fulcrum. It may be noted that several of the tergo-coxal muscles also contribute to this mechanism. Following are the important muscles:

Median dorsal longitudinal muscle (Fig. 8): This is a very wide sheet of muscle whose fibres runs from the second phragmata to the third phragmata. By its action it arches the tergum thus acting as a wing depressor. This beetle being a good flier, the muscle is very well developed.

Oblique tergal muscle (Fig. 8): This muscle takes origin lateral to the dorsal longitudinal muscle and slightly on the posterior side of the metanotum. Its fibres are short and runs back obliquely and are inserted on the posterior phragma below the dorsal muscles. This also acts as an indirect wing depressor.

Dorso-ventral muscles: The important dorso-ventral muscles are the tergo-sternal, and the tergo-coxal muscles of the leg.

Tergo-sternal muscle (fig. 8): This is the most important muscle in relation to the indirect flight mechanism. It takes origin on the anterior part of the scutum as a wide sheet of muscle and runs almost vertical to be inserted on the eusternum in front of the basisternum. It has an antagonistic action to that of the dorsal longitudinal muscle and depresses the tergal wall so as to make the upward wing stroke.

First tergo-coxal muscle (Anterior tergo-coxal muscle) (Fig. 8): This muscle runs just posterior to the tergo-sternal muscle almost parallel to it. It arises on the tergal wall, posterior to the tergo-sternal muscle in the same plane and is inserted on the membrane which joins the basisternum to the coxal wall. This insertion is on a special apodemal cup developed on the articular membrane. This muscle, since the coxal wall has lost its mobility due to the complete fusion with the body wall, has secondarily become an indirect wing levator.

Second tergo-coxal muscle (Fig. 8): This muscle takes origin on the posterior side of the tergum and is inserted deep down in the cavity of the coxa, just above the coxo-trochanteral joint. The coxal wall is modified in this region to form a very big articular cup to receive this muscle. The high development of this muscle, in spite of the complete fusion of the coxa, can only be interpreted as it having undertaken a different function of being a wing levator.

All the three muscles described above lie in the same plane and run almost parallel to each other. They are well exposed by cutting the metathorax in the median longitudinal plane and removing the furcasternum and muscles attached to it.

Direct muscles:

The first basalar muscle (Fig. 9): This muscle has its origin on the basalar sclerite which has been modified to form an apodemal cup for its attachment. The same cup also gives attachment to the second basalar muscle. The first basalar muscle runs vertically downwards lateral to the tergo-sternal muscle andc an be exposed clearly by removing the tergosternal muscle in a lateral dissection. On the lower side it is attached to the sternal wall. The basalar muscle functions as depressor of the costal margin of the wing during flight and as an extensor of the fixed wing and is described as the pronator-extensor muscle of the wing.

Second basalar muscle (Fig. 9): This muscle is attached on the same basalar cup close to the first basalar muscle but runs backwards and is attached to the anterior rim of the coxa.

Subalar muscle (Fig. 9): This muscle takes origin on the lateral margin of the coxa and is inserted on the apodemal cup offered by the subalar sclerite.

All the three muscles, the first and the second basalar and the

subalar, described above, lie in the same plane and can be exposed by removing the first median set of dorsoventral muscles *i.e.* the tergo-sternal and tergo-coxals of the leg.

Coxo-dorsalis metathoracis (Fig. 9): This muscle arises on the scutum and runs as a fine tendon above the subalar muscle and attaches itself to the posterior rim of the coxa.

The axillary muscles are not prominently developed.

Discussion

The view that the dorso-ventral indirect flight muscles might have been primitive coxal muscles, and that these have acquired new function by changing their attachment from the coxal rim to the sternum has been put forward recently. Tiegs (1955) has worked out the flight muscles of several orders of insects and the evidence from Orthoptera suggests that the above view may be correct. Tiegs states further that " The dorso-ventral muscles inserted on the leg base were, even without change of attachments, used in the capacity of indirect wing levators ". This view is more applicable to coleoptera as the leg base is fixed and the coxa has lost its primitive freedom of movement.

Chadwick (1953) states that " The noteworthy modification in some of the modern species is the tendency for the coxal segment of the leg to become incorporated into the body wall ". He further states that this modification which deprives the coxa of its primitive freedom of movement reverses the former origin and insertions of several of the dorso-ventral leg muscles, thus rendering them highly effective as depressers of the tergal margin and hence as elevators of the wings. Observations on the flight muscles of *Sternocera chrysis* var. *chrysidoides* support the above mentioned view. All the dorso-ventral muscles of the metathorax especially those on the coxal rim *i.e.* tergo-coxal anterior and tergo-coxal posterior are highly developed and function as tergal depressors.

A noteworthy point about the endoskeleton in this beetle is that there is no pleural apophysis developed at all, while sternal apophysis or the furcasternum is also poorly developed even in the metathorax. A complicated and highly developed furcasternum as well as the pleural apophysis have been observed in terrestrial beetles like Tenebrionidae and some aquatic beetles. The poor development of apophysis in *Sternocera chrysis* is not surprising as legs have only limited function in this beetle.

Summary

1. The skeleton as well as the musculature of the thorax of the buprestid beetle *Sternocera chrysis* var. *chrysidoides* is described. The great development of the coxal musculature in the metathorax is a noteworthy feature.

2. The observation of Chadwick that the incorporation of the coxa in the body wall enabled a reversion of the original attachments of the dorso-ventral leg muscles thus rendering them effective as tergal depressors, is supported.

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THE MYOLOGY OF THE CHELONIAN LIMB

1. The forelimb of *Lissemys punctata*

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THERE is considerable confusion regarding the homologies of the appendicular muscles in the various tetrapod classes though a good lot of work has been done since the time of Perrin who in 1892 commented in his paper that, "si on ouvre un livre d' Anatomie comparée on est immédiatment frappé du peu de development donné à la myologie comparée." This was reiterated by Romer in 1942. Several valuable contributions have been made by Howell, Appleton, Romer and Haines and our picture of the phylogeny of the appendicular muscles is today much clearer. Compared to mammalian musculature, our knowledge of the muscles of reptiles is very meagre indeed. The situation has remained so because of a general lack of precise myological and neurological information. Among the reptiles, most of the important studies have been done on lizards, Rabl (1916), Straus (1942), Haines (1935 and '50), Romer (1923, '42 and '44), while those on Chelonia are very few, *viz.* Owen (1866), Furbinger (1874 and 1876), Brune (1905), Ogushi, K. (1913), Haines (1939) and Walker (1947). The group, Chelonia, presents a host of anatomical peculiarities and the changes in musculature are not in any way less striking than those of other systems.

The common Indian pond turtle, *Lissemys punctata* has been chosen for the present study. No embyological investigation was however, attempted because we thought it essential to have some basic myological and neurological information which is hitherto not available on this turtle, as a prerequisite to any such attempt. The need for such a study has been further enhanced by the discovery in this turtle of a striated muscular sheath on the lungs (George and Shah, 1954). The presence of such a muscle sheath acting as a pair of bellows in an animal with a hard body shell incapable of body wall movements for respiration, suggests that such respiratory equipment must have given the early chelonians their survival value and also that the Lissemydinae which possess this are a primitive group (George and Shah, 1955).

Muscles of the Pectoral Girdle and Upper Arm :

The *pectoralis* is a broad, fan shaped muscle having its origin on the plastron and along the margin where the carapace joins the plastron at the lateral boundary. The origin of this muscle is the same as in other reptiles and mammals considering that the plastron of Chelonia comprises the modified sternum and the clavicles. All the fibres of the muscle run obliquely forward and converge towards the lesser tuberosity on the proximal end of the humerus, where it gets inserted by a thick tendon. It is innervated by a branch of the first thoracic and also by a branch from the brachialis inferior nerve of the brachial plexus. The muscle acts as a powerful adductor of the arm.

The *deltoideus* (Fig. 1) arises by two heads, a clavicular one from the epiplastral region of the plastron and the other, the scapular arising from the outer surface of the acromion process of the scapula (precoracoid). Finally both the heads join together and gain a common insertion on the bony protruberance near the head of the humerus. In the case of the spiny tailed lizard, Uromastix, the scapular part of the *deltoideus*, however, arises from the suprascapula (George, 1948). In lizards the primitive *deltoideus* during the course of its evolution has given rise to three distinct heads, the clavicular, the scapular and the third which is the *scapulo-humeralis anterior* muscle (Romer, 1944). The presence of only the first two, in Lissemys, probably denotes that the third one *(scapulo-humeralis anterior)* has not been separated out as in the lizards. If this supposition is true then the *deltoideus* of this animal is more primitive than that of the lizards. It is a powerful abductor of the arm with the two heads of the muscle separately innervated by branches of the deltoid nerve.

On reflecting the above muscles the second layer of muscles on the ventral side consisting of the *biceps*, the *coracobrachialis* and the *supracoracoideus* is exposed.

The *biceps* (Figs. 1 & 2) arises by two fleshy heads from the posterior border of the coracoid and proceeds in two bellies one being larger and longer than the other. The larger, the *biceps superficialis* (Figs. 1 & 2) arises from the posterior border of the coracoid at its free end, while the smaller one, *biceps profundus* (Figs. 1 & 2), arises from the proximal part of the same border of the coracoid. The two bellies run laterally and gain separate insertions. The *biceps profundus* after running for a short distance becomes thin, chord-like and tendinous and gets inserted along with that of

brachialis inferior on the proximal part of the radius and ulna, where both of them are closely apposed to one another. The common insertion of *biceps profundus* and *brachialis inferior* is the only indication that the posterior part of the *brachialis inferior* was united with the *biceps profundus* during development (Walker, 1947). The *biceps superficialis* shows a slight tendinous constriction on its fleshy belly and finally becomes tendinous as it proceeds outwards. The tendon is partly ensheathed along with that of *biceps profundus* in a common connective tissue covering. The *biceps superficialis* gets inserted on the distal half of the radius and also on the radiale. In Crysemys, the *biceps superficialis* consists of two fleshy parts separated by a conspicuous tendinous part but there is no such prominent separation of the two in Lissemys but as already stated only a thin tendinous intersection is present. In Uromastix, however, both the heads of *biceps* have a common insertion on the upper end of the radius (George, 1948). The *biceps* in Lissemys acts as the flexor of the forearm and the adductor of the arm and to a limited extent the manus also. Both the bellies of the muscle are innervated by separate branches which arise from the brachialis inferior nerve of the brachial plexus.

The *supracoracoideus* (Fig. 1) arises from the entire ventral surface of the coracoid and also the posterior border of the precoracoid. The fibres of these two separate heads decussate and finally run outwards and get inserted on the lesser tuberosity of the humerus. The muscle occupies the major part of the fenestra bounded by the precoracoid, coracoid and acromio-coracoid or the precoraco-coracoid ligament. It is an adductor of the arm. The muscle is innevated by supracoracoideus nerve.

The *coraco-brachialis brevis* (Figs. 1 & 2) is a small muscle, just under cover of the *supracoracoideas*, in front of the *biceps profundus* in early stages of development but later gets separated. It arises from the posterior border of the coracoid at its proximal end just in front of the origin of the *biceps profundus*, runs outwards and gets inserted on the hollow between the two tuberosities at the proximal end of the humerus. Owen has named this muscle as *teres minor*. It acts as an adductor of the arm. A branch of the brachialis inferior nerve innervates this muscle.

The dorsal group of muscles that have attachments to the shoulder girdle consists of the *latissimus dorsi*, the *teres major*, the *subscapularis*, the *coracobrachialis magnus*, the *serratus magnus*, and the *subclavius*.

The *coracobrachialis magnus* (Fig. 2) (*subcoracoideus* of Owen, 1866;

Noble and Noble, 1940) arises from the entire dorsal surface of the coracoid and also some part of the dorsal surface of the acromio-coracoid ligament. It runs outwards and gets inserted on the greater tuberosity of the humerus. It acts as an adductor of the arm. In lizards, too, this muscle is the most prominent muscle on the dorsal side in the coracoid region. In crocodile, however, it is absent (Romer, 1944). This muscle is innervated by the nerve coracobrachialis magnus which arises from the brachialis inferior nerve.

The *latissimus dorsi* (Fig. 2) has a fleshy origin on the carapace at the first costal plate near the vertebral column. After taking a broad origin, the muscle runs outwards and narrows down into a flat, short tendon which gains insertion on the outer side of the neck of the humerus. This muscle in other reptiles like lizards and crocodiles is flat and fan shaped arising from the spines of the first few thoracic vertebrae or the fascia covering this region. It acts as a retractor and an adductor of the upper arm. A branch from the deltoid nerve innervated this muscle.

The *teres major* (Fig. 2) which is an adductor of the upper arm and forms a large muscle mass on the antero-dorsal side of the scapula arises from the anterior border and the medial surface of the proximal end of the scapula and the suprascapula. It runs laterally towards the humerus and finally gains insertion on the dorsal side of the neck of the humerus. This muscle runs side by side with the *latissimus dorsi* during development and later as the adult stage is reached, gets separated at the point of its origin but still shows a somewhat common insertion, on the neck of the humerus. In Uromastix, (George, 1948) and other lizards the muscle is absent, which denotes that it is a special acquisition in Chelonia among reptiles and must have remained as a part of the *latissimus dorsi* in the other reptiles. In turtles this separation of the *teres major* from the *latissimus dorsi* has been necessitated by the modification in the skeleton and the limb movements. A separate *teres major* is also present in the birds and mammals and must have made its appearance as a result of the increased scope of the limb movements. This muscle is innervated by a branch from the deltoid nerve.

The *subscapularis* (Fig. 2) is the most massive muscle on the postero-lateral side of the scapula. It arises from the antero-dorsal and the dorso-posterior border of the suprascapula and also from the entire dorsal and postero-ventral surface of the scapula. All the fibres from these different

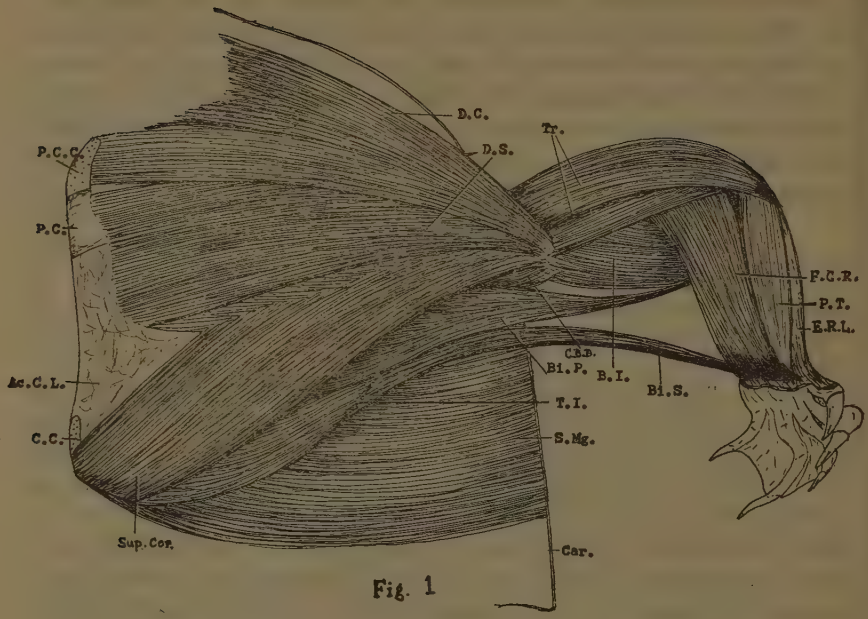
parts after their origin run laterally and converge near the dorsal rim of the glenoid cavity, where the muscle becomes tendinous and gets inserted by the side of the insertion of the *coracobrachialis magnus*, on the outer tuberosity of the proximal end of the humerus. It acts as an adductor and to some extent as a lateral rotator of the arm. It is innervated by the subscapular nerve which arises from the dorsal ramus of the seventh cervical nerve.

The *subclavius* (Fig. 2) arises from the under surface of the first costal plate and gets inserted on the suprascapula by a thick tendon. It keeps the girdle in position. One of the branches which arises from the ventral ramus of the first thoracic innervates the muscle.

The *serratus magnus* (Figs. 1 & 2) is a flat sheet of muscle on the dorsal side of the shoulder girdle. It has a long linear origin from the antero-lateral border of the carapace where the carapace gets attached to the plastron. The posterior half of the muscle runs medially and finally gains a fleshy insertion on the entire antero-dorsal border of the coracoid, while the anterior half of the muscle gets inserted on the postero-ventral border of the scapula. The muscle acts as the depressor and the lateral rotator of the shoulder thereby bringing about the abduction of the arm. The action of this muscle in respiration bringing about inspiration has been demonstrated by McCutcheon (1943). A branch from the ventral nerve cord of the brachial plexus innervates the muscle.

The *triceps* (Figs. 1 & 2) muscle completely covers the dorsal side of the humerus. It arises by three separate heads, the dorsal one from the antero-dorsal part of the rim of the glenoid cavity, the second which is just under cover of the first, arises from most of the dorsal surface of the humerus, while the third from most of the ventro-medial surface of the humerus. The first two heads unite together and form a thin flat tendon which gets inserted on the outer surface of the olecranon process of the ulna, while the third one gains its insertion separately by a narrow tendon on the outer condyle of the proximal end of the ulna. The first two heads of the *triceps* act as an extensor, while the third as the flexor of the forearm. Three different branches from the triceps nerve, which arises from the brachialis superior nerve in turn, innervate the three heads of the triceps respectively.

The *brachialis inferior* (Figs. 1 & 2) is a fan shaped muscle having a broad origin and arising from the proximal half of the humerus at the


D.C.
Tr.
P.C.C.
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C.B.B.
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B.I.
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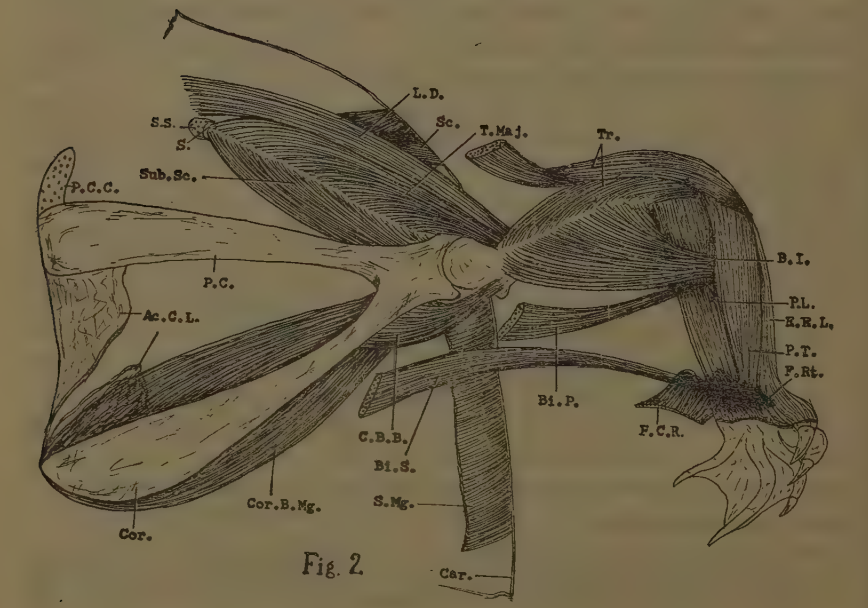
Ac.C.L.
T.I.
C.C.
S.Mg.
Sup.Cor.
Car.

Fig. 1


L.D.
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Ac.C.L.
E.R.L.
P.T.
F.Rt.
Bi.P.
C.B.B.
F.C.R.
Bi.S.
Cor.B.Mg.
S.Mg.
Cor.
Car.

Fig. 2

deltoid ridge. The fibres of the muscle converge to form a thick tendon which finally gets inserted on the ulna. The insertion of this muscle is along side with that of *biceps profundus*. The *brachialis* acts as a flexor and a lateral rotator of the forearm. The brachialis inferior nerve sends out a branch to innervate this muscle.

Muscles of the Forearm and Hand

The dorsal muscles of the forearm in Lissemys present an interesting arrangement distinct from the general pattern and disposition of muscles found in other reptilian groups. These changes in the arrangement of the muscles are essentially due to the shifting of the ulna to the dorsal side of the radius. The extensor muscles occur in two layers with *external radialis longus* ; the *external radialis brevis* (*extensor carpi-radialis brevis*) ; the *external ulnaris* ; the *supinator* and the *external digitorum communis* forming the upper layer and the *extensor digitorum profundus* forming the deep one.

The *external radialis longus* (Fig. 3, B) is a composite muscle formed of the *branchio-radialis*, the *extensor pollicis* and the *extensor indicis* all having a common origin from the inner condyle of the humerus at its distal end. More or less at the level of the proximal carpals the muscle is differentiated into three slips. The medial one called *extensor indicis* runs towards the lateral side of the index finger and gets inserted on the base of its proximal phalanx along with the slip of the *extensor digitorum communis* to this digit. The middle slip which is the *extensor pollicis* runs on the dorsal side of the thumb and gains a fleshy insertion on the dorsal side of the proximal end of the proximal phalanx of the thumb. The third, the lateral one, which is the most massive of all the three, gets inserted on the inner side of the first phalanx of the thumb and forms the *brachio-radialis*. The *brachio-radialis* in mammals has the same source of nerve

Figs. 1 and 2 : Muscles of the pectoral girdle and upper arm of Lissemys. (Pectoralis muscle is completely removed) Ventral view.

Ac. C. L., Acromio-coracoid ligament; B. I., Brachialis internus; Bi. P., Biceps profundus ; Bi. S., Biceps superficialis ; Car., Carapace ; Cor., Coracoid ; C. B. B , Coracobrachialis brevis; Cor. B. Mg., Coracobrachialis magnus; C. C., Coracoid cartilage; D.C., Clavicular head of the deltoideus; D. S., Scapular head of the deltoideus; E.R.L., External radialis longus ; F. C. R., Flexor carpi radialis ; F. Rt., Flexor ratinaculum ; L. D., Latissimus dorsi ; P. C., Precoracoid ; P. C. C., Precoracoid cartilage ; P. L., Palmaris longus ; P. T., Pronator teres ; S., Scapula ; Sc , Subclavius ; S. S., Suprascapula ; Sup. Cor., Supracoracoideus ; Sub. Sc., Subscapularis ; S. Mg. Serratus magnus ; Tr. Triceps; T. I., Tendinous intersection on the biceps superficialis ; T. Maj., Teres Major.

supply as the extensor groups of muscles and so it is probable that this muscle is actually derived from the dorsal group of muscles evidently as a result of the shifting of the ulna as already mentioned. Moreover it is not a completely separate muscle but has a common origin with the *extensor pollicis* which is a part of the extensor group of muscles. The *external radialis longus* is innervated by a branch from the triceps nerve.

The *external radialis brevis* (*Extensor carpi-radialis brevis*) (Fig. 3, A) arises as a very thin tendinous strip from the lateral condyle of the distal end of the humerus. It runs towards the thumb and on its way joining with the fibres arising from entire outer border of the radius gets inserted on the radiale. It acts as an extensor of the arm and lateral rotator of the first digit. A branch of the radiale nerve innervates this muscle.

The *supinator manus* (Fig. 3, A) is a small extensor muscle. It arises from the lower part of the shaft of the ulna and gets inserted on the base of the first metacarpal. Haines (1939) has reported this muscle in Emys as a peculiar muscle not found in other chelonians studied by him and by other workers. The muscle is a supinator of the manus and is innervated by the ulnar nerve.

The *extensor digitorum communis* (Fig. 3, B) arises from the dorso-lateral side of the outer condyle of the distal end of the humerus and carries fibres arising from the outer side of the shaft of the ulna. Such an origin from ulna also is rather unusual but has taken place due to the shifting of the ulna dorsalward. As the muscle approaches the manus and comes to the level of the distal end of the ulna it presents a tendinous intersection, from where the muscle proceeds in four fleshy slips each of which gets inserted on the outer side of the second to the fifth metacarpals respectively and the base of the respective proximal phalanges. The first digit which has an extensor of its own does not receive a slip from this muscle. The insertion of this muscle slips on the lateral sides of the metacarpals and the base of their respective proximal phalanges is a specialization for the powerful outward beat of the hand in swimming. It acts as a powerful extensor and a lateral flexor of the manus. A branch from the triceps nerve innervates this muscle.

The *external ulnaris* (Fig. 3, B) is a well developed muscle situated on the outer side of the forearm. It consists of two component muscles, which are separated only towards the insertion, into an extensor part

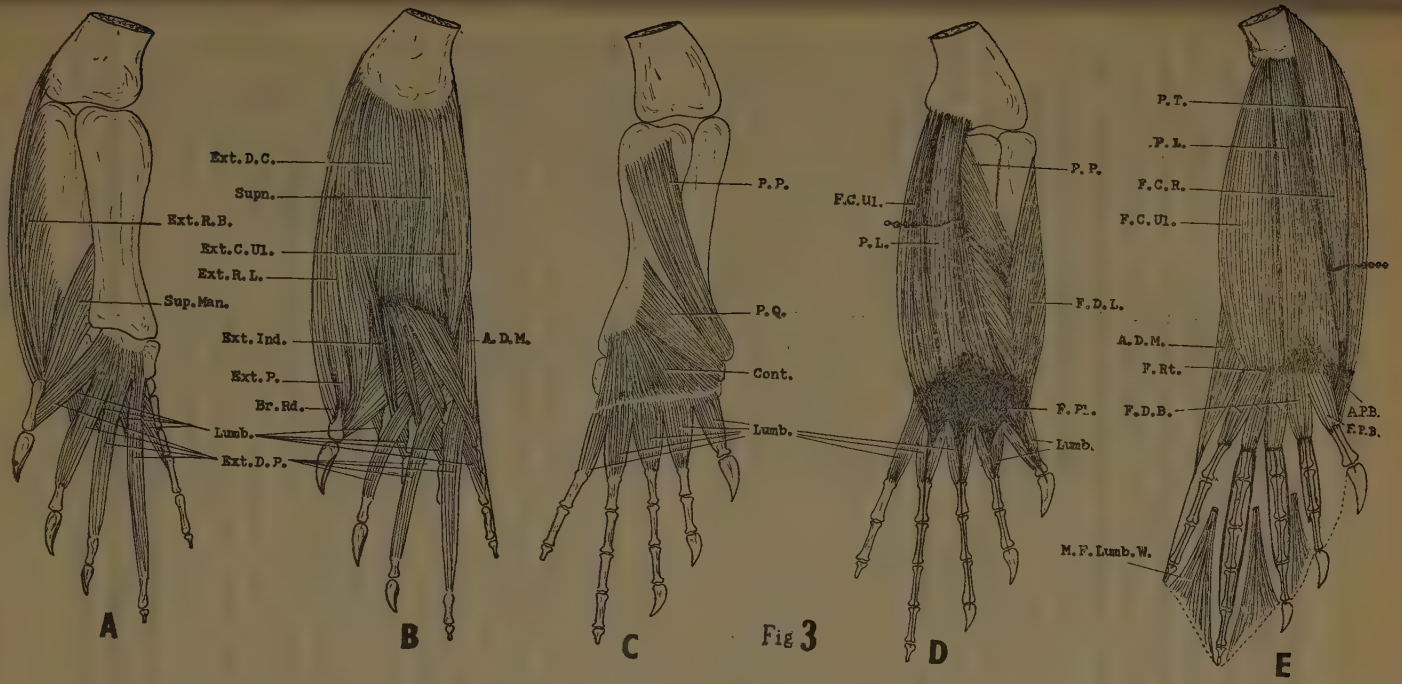

Fig. 3 (A and B) Deep and superficial muscles on the dorsal side of the forearm and hand of Lissemys.
(C, D and E) Deep and the superficial muscles on the ventral side of the forearm and hand of Lissemys.

A. D. M., Adductor digiti minimi; A. P. B., Adductor pollicis brevis; Br. Rd., Brachioradialis; Cont., Contrahentes; Ext. C. Ul., Extensor carpi ulnaris (Extensor part of the external ulnaris); Ext. D. C., Extensor digitorum communis; Ext. D. P., Extensor digitorum profundus; Ext. Ind., Extensor indicis; Ext. P., Extensor pollicis; Ext. R. B., Extensor radialis brevis; Ext. R. L., Extensor radialis longus; F. C. R., Flexor carpi radialis; F. C. Ul., Flexor carpi ulnaris (Flexor part of the external ulnaris); F D. B., Flexor digitorum brevis; F. D. L., Flexor digitorum longus; F. Pl, Flexor plate; F. P. B, Flexor pollicis brevis; F. Rt., Flexor ratinaculum; Lumb., Lumbricles; M. F. Lumb. W., Muscle fibres of lumbricles to the web; P. L., Palmaris longus; P. P., Pronater profundus; P. Q., Pronater quadratus; P. T., Pronator teres; Supn., Supinator; Sup. Man., Supinator manus.

dorsally (the *extensor carpi-ulnaris*, Fig. 3, B) and flexor part ventrally (the *flexor carpi-ulnaris*, Fig. 3, D and E). This common muscle mass arises from the outer condyle of the distal end of the humerus and remains as a single belly upto the distal end of the ulna where it bifurcates. The dorsal of the two gets inserted on the distal end of the ulna and the dorso-lateral side of the ulnare, while the other runs on the ventral side of the ulna and the ventro-lateral side of the ulnare where it gets inserted. Such a split in the composition of the muscle is a specialization for the paddling movement of the forearm in swimming. This muscle mass is innervated by the ulnar nerve which arises from the brachialis inferior nerve.

The *supinator* (Fig. 3, B) which is a powerful muscle does not act as the supinator of the other vertebrates but as a powerful extensor of the forearm. It arises from the dorsal surface of the humerus at its distal end and also from the outer surface of the proximal end of the ulna and gains a tendinous insertion on the fused intermedium and centrale. In the natural course of events the insertion of this muscle should have been on the radius but owing to the ulna having shifted to a position dorsal to the radius, it has acquired the present insertion. A branch of the triceps nerve innervates this muscle.

The *extensor digitorum profundus* (Fig. 3, A & B) arises from the fused intermedium and centrale. It divides itself into four muscular slips and each one runs forwards, towards the digits, where the first slip goes to the thumb and gains insertion on the medial side of the proximal phalanx of the thumb. The second one is inserted on the dorsal side of the middle phalanx of the second digit. The third and the fourth are inserted on the dorsal side of the proximal end of the fourth phalanx of the third and the fourth digits respectively. The last digit does not receive any muscular slip from the *extensor digitorum profundus*. The muscle acts as an extensor of the manus as a whole and also of the first four digits in particular through the respective digital slips. It must be mentioned, however, that owing to the insertion of the first slip on the medial side and then on the dorsal side of the proximal phalanx of the thumb, the muscle is more of an adductor of the thumb than the extensor. The muscle is innervated by the triceps nerve.

On the flexor side of the forearm the superficial layer consists of the *flexor carpi-ulnaris*, the *flexor carpi-radialis* and the *palmaris longus*; while in the hand the *flexor digitorum brevis* forms the most superficial muscle.

Palmaris longus (Fig. 3, D & E) is one of the massive muscles on the flexor side of the forearm. It arises from the outer condyle of the distal end of the humerus just medial to the origin of the *flexor carpi-ulnaris* and also from the outer margin of the ulna. The fibres run downwards towards the carpals. When they reach the level of carpals they form a thin tendinous plate which gets inserted on the flexor plate formed by the *flexor digitorum longus*. It is innervated by a branch of the median nerve. The muscle acts as a flexor of the hand.

The *pronator teres* (Fig. 3, E) which as the name indicates acts as a pronator of the forearm. It arises from the inner condyle of the distal end of the humerus. It has a broad origin with the fibres later converging towards the distal end of the radius to be inserted on the radius and the radiale. It is innervated by a branch of the median nerve.

The *flexor-carpi-radialis* (Fig. 3, E) is a very well developed muscle arising by a fleshy origin from the outer margin of the distal part of the humerus and gets inserted on the distal end of the radius by a thick tendon and by a thin, flat tendon on the tendinous distal part of the *biceps superficialis* muscle. A branch of the median nerve innervates this muscle.

At the distal end of the radius on the flxor side there is a thin tendinous transverse plate, the flexor retinaculum covering the distal ends of the *flexor carpi-radialis, pronator teres* and *flexor carpi-ulnaris*.

The *flexor digitorum brevis (flexor brevis superficialis*, Haines, 1950) (Fig. 3, E) arises from the flexor ratinaculum and the palmar aponeurosis and soon differentiates itself into four short fleshy slips which go, one each to the second to the fifth digits respectively. The slips corresponding to the second, third and fourth are further differentiated into a pair of thin tendinous parts which run on either side of their respective digits to be inserted on the proximal end of the last but one phalanx. The small thin slip of the fifth digit, however, does not show any such differentiation and its thin tendon gets fused with the main flexor tendon. A branch of the median nerve innervates this muscle.

The *flexor pollicis brevis* (Fig. 3, E) arises from the flexor ratinaculum and gets inserted on the proximal end of the proximal phalanx of the first digit. It is innervated by the branch from the median nerve.

The *abductor pollicis brevis* (Fig. 3, E) is a small muscle arising from the radiale and gets inserted on the lateral border of the first

metacarpal and the proximal end of the proximal phalanx of the first digit. It is innervated by the terminal branch of the triceps nerve.

The *abductor digiti minimi* (Fig. 3, B & E) arises partly from the outer side of the fascia on the distal end of the *external ulnaris* (the composite muscle formed by the *extensor carpi-ulnaris* and the *flexor carpi-ulnaris*) and partly from the flexor retinaculum. Its fibres then converge and finally get drawn out into a thin long tendon which gets inserted on the outer side of the terminal phalanx of the last digit. It acts as an abductor of the last digit. The ulnar nerve after taking a turn on the dorsal side gives out a branch to innervate this muscle.

The *abductor pollicis brevis*, the *flexor pollicis brevis* and the *abductor digiti minimi* belong to the *flexor brevis superficialis* group of muscle (*flexor digitorum brevis* Haines, 1950).

The deep layer of the flexor muscles consists of the *flexor digitorum longus*, *pronator quadratus* and the *pronator profundus*.

The *flexor digitorum longus* (Fig. 3, D) in Lissemys consists of two heads, unlike in the monitor lizard, Varanus where it consists of five heads (Haines, 1950) of which the three arising from the humerus, are the *condylo-radialis*, *condylo-ulnaris*, *centralis* and the other two of which one arises from the shaft of the ulna and the other from the carpal, the ulnare. The one arising from the shaft of the ulna, in Varanus, forms a broad and thick flexor plate to which the three parts arising from the humerus are attached. In Lissemys, one of the two heads of the muscle arises from the ventral side of the shaft of the ulna and the other from the ventral side of the shaft of the radius. The fibres of both the heads decussate and finally run towards the carpus where at the level of the distal end of the radius, ulna and the carpals these fibres form a well-developed tendinous plate, the flexor plate, which later divides into four thick tendons each of which runs towards its corresponding digit (the first four digits). After traversing through the ligamentous tunnel at the joints of the phalanges each one gets inserted on the proximal end of the terminal phalanx of its respective digit. This muscle is one of the most powerful flexors of the digits. The presence of only a couple of heads for the *flexor digitorum longus* in Lissemys appears to be a degenerate condition, as the animal having considerably lost the prehensile ability of the hand. These muscle heads may therefore be regarded as corresponding to the deep, massive

muscle referred to by Haines (1950) as arising from the shaft of the ulna in Varanus. A branch of the median nerve innervates the muscle.

The *pronator profundus* (Fig. 3, c) is a very powerful pronator of the forearm and manus which arises as a broad massive muscle belly from the proximal end of the ulna and runs obliquely downwards narrowing down to be inserted on the adjacent surface of the carpals and by a thin tendon which goes further to get inserted on the proximal phalanx of the first digit. The muscle is innervated by a branch from the median nerve.

The *pronatar quadratus* (Fig. 3, c) is a small pronator muscle situated just under cover of the *pronator profundus*. It arises from the lower half of the medial surface of the ulna and is inserted on the carpal and the distal end of the radius. The median nerve innervates this muscle.

The *contrahentes* (Fig. 3, c) belonging to the first and the last digits arise from the ulnare and radiale respectively, while those of the second, third and fourth digits arise from the dorsal surface of the flexor plate. All of them proceed towards their respective digits and finally get inserted on the proximal end of the second phalanx of their respective digits. These muscles are quite powerful and act as the flexors of the digits. Branches of the median nerve innervate these muscle bellies.

The *lumbricals* (Fig. 3, A, B, C & D) arise from the carpals and proceed in four pairs towards the second, third, fourth and the fifth digits. The couple on reaching the digit separate and run forwards independently along the sides of the respective digits and gain their insertions on the base of the subterminal phalanx. There is only one *lumbrical* on the medial side of the fifth digit. It is interesting to note that some fibres of the lumbricals get inserted on the web (Fig. 3, E) also thereby rendering the webbed manus a more efficient organ for swimming. These muscles are also innervated by the branches of the median nerve.

On the lateral side of the second phalanx of the second, third, fourth and fifth digits, the *interossii* muscles occur as small muscle bundles which arise from the second phalanx and get inserted on the proximal end of the next adjacent phalanx. Branches from the median nerve innervate these muscles.

Summary

1. The muscles of the shoulder girdle and forelimb of the pond turtle, *Lissemys punctata*, are described.

2. The major changes in the attachments, disposition and action of the muscles have been effected by the shift of the ulna dorsalward.

3. The *supinator* instead of having its normal function, acts as a powerful extensor of the manus.

4. The *external ulnaris* which arises as a single muscle but later splits into two, is a unique one whose one part acts as a flexor (*flexor carpi-ulnaris*) and the other an extensor (*extensor carpi-ulnaris*).

5. The presence of the primitive *supinator manus* further supports the contention that *Lissemydinae* are a primitive group.

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STUDIES ON THE STRUCTURE AND PHYSIOLOGY OF THE FLIGHT MUSCLES OF BATS

1. The Occurrence of Two Types of Fibres in the *Pectoralis Major* Muscle of the Bat (*Hipposideros speoris*), Their Relative Distribution, Nature of the Fuel Store and Mitochondrial Content

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[1 Plate]

George and Naik (1957a and 1957b) established the existence in the *pectoralis major* muscle of the pigeon, two fundamentally distinct types of fibres, a white, glycogen-loaded, broad variety and another red, fat-loaded, narrow one, the latter having a considerably larger quota of mitochondria than the former (1957c). From the work of George and his school (George, 1952; George and Jyoti, 1953, 1955a, 1955b, 1957; George and Scaria 1956, 1957; George and Naik, 1957a, 1957b, 1957c) it has become evident that fat is the chief fuel for long and sustained muscular activity in flying birds and that the development of the red, narrow, fat-loaded variety of fibres in the breast muscle seems to be a clear case of adaptational differentiation. The bird and bat are both flying animals indulging in sustained muscular activity and it was thought desirable to make similar studies on the pectoral muscle of the bat (*Hipposideros speoris*) and hence the justification of these studies.

Material and Methods

The animal was decapitated and pieces of the *pectoralis major* muscle were cut out for the different preparations. For general observations, fresh frozen sections were cut and mounted in isotonic sucrose solution. For the measurement of the diameter of the fibre the preparations were made in the same way except that the muscle piece was removed from the killed animal only after it showed no response to external stimulus. For the study of the distribution of the fibres, the sections were projected on the screen of a microphotographic camera and exposed directly on the photographic printing paper. For the measurement of the diameter of the fibres the sections were observed under oil-immersion and the diameter measured using a scaled ocular and a micrometer slide.

Fresh frozen sections treated with Janus Green B in isotonic solution to stain mitochondria were mounted in isotonic sucrose solution and immediately photographed.

To demonstrate lipids in the fibres, frozen sections of the piece of muscle, fixed in Baker's calcium-formol for a week and embedded in gelatin, were cut and stained with Sudan Black (saturated solution in 70% alcohol).

Paraffin sections of the muscle pieces fixed in Rossman's fluid at O°C were stained with Best Carmine to stain glycogen. The control sections were incubated in saliva. Prior to staining all the sections were coated with celloidin.

Observations

The pectoralis major muscle arises from the keel-like expansion of the body of the sternum and the fibres run laterally and forwards converging in a flat tendon, which is inserted at the medial base of the deltoid ridge. Anteriorly, the muscle is thick but more posteriorly it becomes thinner, and thinnest towards the posterior border. The transverse sections of the muscle show that there are two distinct types of fibres as in the pigeon breast muscle. The fibres in the interior of the muscle consists predominently of the narrow ones (the ratio of broad to narrow being 1 : 10) while in the superficial part (fig. 1) there is a gradual increase of the broad fibres, with the result that the broad ones are comparatively more there (the ratio of broad to narrow being 1 : 2). In the posterior part of the muscle the narrow fibres are not so numerous as in the anterior region.

The marginal position of the broad fibres in a fasciculus is maintained only in the superficial region of the muscle (fig. 1) even though not so distinctly as in the pigeon. The broad (white) fibres have a diameter of 59μ while the narrow (red) ones 39μ. (These values represent the mean diameter of a large number of fibres.)

In the section stained with Janus green B, the mitochondria are considerably more numerous and larger in the narrow fibres than in the broad ones (fig. 3). In the narrow fibres numerous unstained fat globules of various types are also visible. It was also noticed that, in the fresh frozen sections the broad fibres present more and larger ice crystals in them than the narrow ones.

In preparations stained with Sudan Black the mitochondria appear lightly stained whereas the fat globules are deeply stained. In the white fibres the fat gloubles are extremely sparse and to the contrary in the red ones (fig. 4). The fat gloubles are disposed in longitudinal rows between the areas of Cohnheim but they do not however seem to be associated with either the transverse bands of the fibre or the mitochondria.

Those sections stained for glycogen show that all broad fibres without exception, possess a much higher concentration of glycogen in them than the narrow ones (fig. 2). The staining was uniform and no polarisation of glycogen due to fixation was observed.

Discussion

The occurrence of red and white fibres in a single muscle is well known and a greater affinity of the former for Sudan dyes has been noted by several workers. George and Naik (1957 b & c) showed that in the *pectoralis major* muscle of the pigeon, the white fibres are broad and glycogen-loaded, whereas the red ones, narrow and fat-loaded and that the latter have a much higher mitochondrial content than the former. In this respect the structure of the *pectoralis major* of the bat (*Hipposideros speoris*) resembles that of the pigeon.

Since Bell (1911) reported and later confirmed by Bullard (1912) that fat globules in muscle fibres disappear on starvation, this interstitial fat was considered solely to be reserve food. George and Jyoti (1955a) demonstrated that the fat-globules in red fibres get depleted during strenuous exercise, George and Scaria (1957) histo-chemically demonstrated in the *pectoralis major* muscle of pigeon and bat (George, Sushila and Scaria, 1957) high lipase activity in the narrow fibres. These findings have thrown some new light on the role of fat globules in muscle fibres. The fat in the muscle has now to be regarded not only as reserve food but also as fuel store, which could be utilized directly for muscular exercise.

On the other hand the white fibres with higher glycogen store and insignificant amount of fat seems to rely wholly on their glycogen store for the production of energy. This clearly means that in a single muscle, two types of cells, *viz.* the glycogen-loaded and the fat-loaded ones have been developed for storing the respective fuels, so that it can efficiently utilize both. It is also indicated that they differ in their enzyme systems, particularly those involved in the building up and the subsequent utiliza-

tion of the two types of metabolites. This assumption finds support in the fact that the two types of fibres markedly differ in their mitochondrial content. In studying these two types of fibres we are faced with the problem as to whether these fibres differ in the mode of their contraction and the findings have also opened out new avenues for further investigation.

Bullard (1912) and recently George and Jyoti (1955 a) reported the presence of only one, the red type of fibres in the breast muscle of bat. Our present study has shown the presence of two distinct types of fibres as in the pigeon breast muscle. We (1957) reported similar differences in the breast muscle of different birds and our observations on the bat breast muscle lead us to believe that such variations as reported in the case of birds also exist in different bats. So there is need for detailed studies on the flight muscles of bats and such are in progress in our laboratories, the present one being the first in the series.

Summary

1. The *pectoralis major* muscle of the bat (*Hipposideros speoris*) consists of two distinct types of fibres, a white, glycogen-loaded, broad variety possessing few mitochondria and a red, fat-loaded, narrow variety having a considerably larger mitochondrial content.

2. Comparison is drawn between these fibre types and those of the pigeon breast muscle.

Acknowledgement

The latter author (R. M. N.) is indebted to the Govt. of India for the award of a Senior Research Scholarship which made these studies possible. Our thanks are due to Mr. V. K. Chari, Curator of the Natural History Section of the Prince of Wales Museum, Bombay, for kindly identifying the bat.

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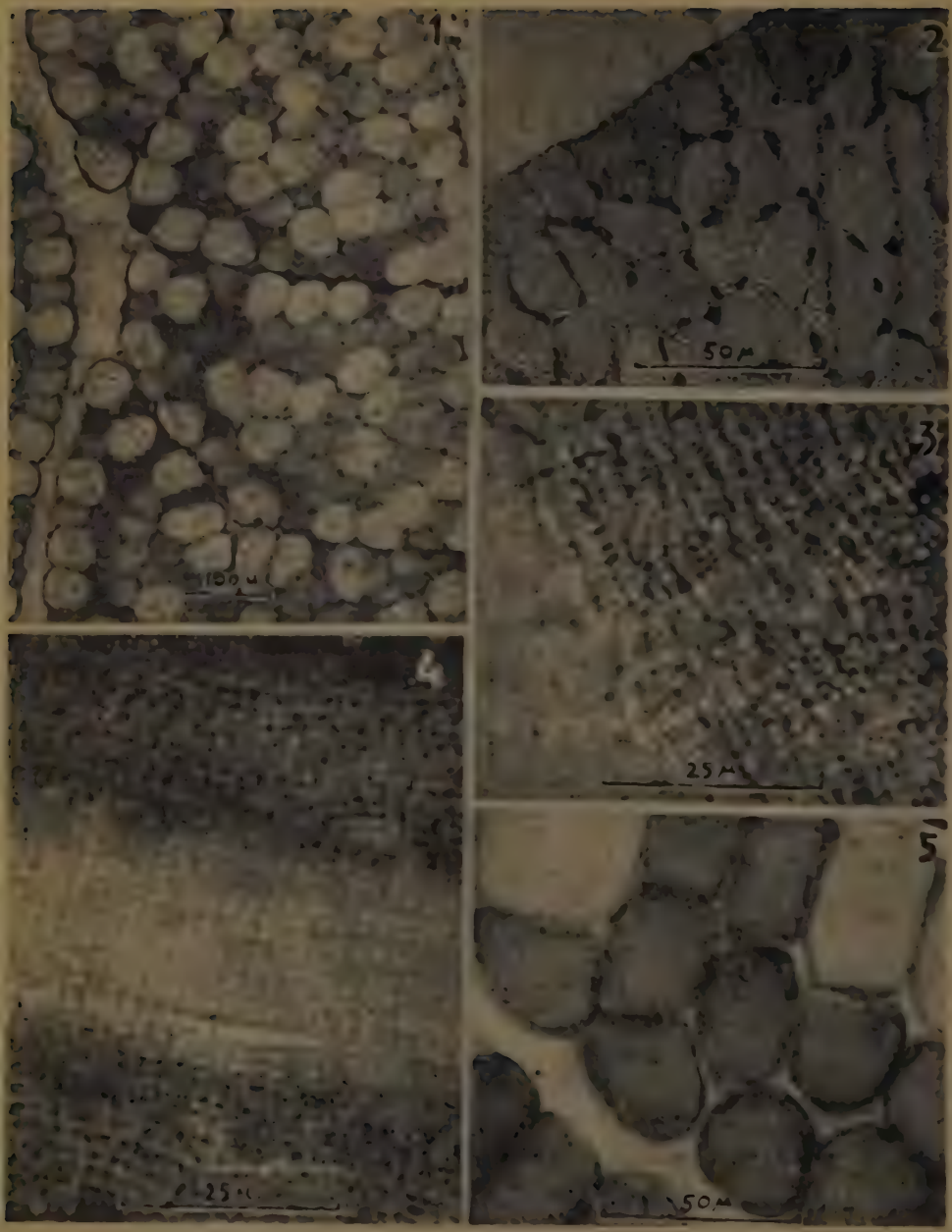
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Plate showing some structural features of the *pectoralis major* muscle of the bat (*Hipposideros speoris*).

Fig. 1 Untreaed T.S. of the superficial region of fresh frozen muscle showing two types of fibres, the broad and narrow ones.

Fig. 2 T.S. of the muscle embedded in paraffin is stained with Best carmine and hemalum. Two white broad fibres on the outer border of the fasciculus and a portion of a third similar fibre on the right uniformly stained, show a considerable larger amount of glycogen in them than the others.

Fig. 3 L.S. of fresh frozen tissue stained with Janus Green B. A portion of the white broad fibre with few mitochondria is seen on the left, whereas on the right is seen the red narrow fibre showing numerous deeply stained mitochondria and unstained refractile fat globules. Being a frozen section, the muscle fibres have contracted considerably, and this can be realised on comparing the distance between the dark bands in the white fibre in fig. 4 where the muscle piece was fixed after stretching it to its resting length on a filter paper, so that the alteration in its length was negligible.

Fig. 4 L.S. stained with Sudan Black, showing an unstained white fibre in between two deeply stained red fat-loaded ones. In the former only lightly stained mitochondria situated at the dark bands are seen, whereas in the latter the deeply stained fat globules are seen prominently and distinctly from the lightly stained mitochondria.

Fig 5 T.S. embedded in gelatin and stained with Sudan Black. Parts of three white fibres are seen on the top. The red ones show greater concentration of Sudan Black. The areas of Cohnheims in the red fibres are lightly stained. In between these areas the muscle columns are deeply stained due to lipoid inclusions and show up in the photograph as dark dots. The white fibres are practically unstained and the muscle columns are hardly visible.

GLYCOGEN CONTENT OF *ACTINOTROCHA* LARVA

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THERE is need for investigations on the glycogen content of embryos and embryonic tissues, for, as Lee (1951) has remarked, it is an open question whether the carbohydrate metabolism in embryonic life is the same as in the adult. There have been relatively few investigations so far on this problem. The glycogen content of chick embryos during different stages of development was studied by Sakuragi (1917), Idzumi (1924), Murray (1925) and other authors. Cytochemical studies on the glycogen of the tissues of frog embryos have been made by Woerdemann (1933) and direct microchemical studies by Heatley and Lindahl (1937), Jaeger (1945). The investigations on glycogen content have, however, been confined almost to vertebrate embryos. Ludwig (1949) has recently studied the glycogen content in the pupal and metamorphosing stages of Japanese beetle (*Popillia japonica*). Lindberg (1945) investigated the metabolism of glycogen in the fertilization of sea urchin egg.

Apart from these investigations, practically no information is available regarding the glycogen utilization in the wide variety of larval forms met with among invertebrates. The present study relates to the utilization of glycogen in the development of *Actinotrocha* larva of *Phoronis*. A histochemical determination of glycogen was made in three different developmental stages of the *Actinotrocha* larva : I. before metamorphosis ; ii. commencement of metamorphosis ; iii. after metamorphosis.

Material and Methods

The *Actinotrocha* larva were obtained from plankton. Three different stages were separated : *viz.* (i) before metamorphosis, (ii) commencement of metamorphosis and (iii) post-metamorphosis. They were fixed in picroformalin in absolute alcohol (9 volumes of absolute alcohol saturated with picric acid and 1 volume of neutralised formalin) for 24 hours. The material was washed in absolute alcohol treated with xylol and embedded in paraffin, taking care not to overheat the tissues as otherwise the glycogen in the tissues might be autolysed. Sections of 8 microns thickness were cut and deparaffinised in xylol and brought upto 90%

alcohol. A treatment with 4% chromic acid was given for nearly an hour and washed in running water for 10 to 15 minutes. After washing, the slides were placed in Bauer's Feulgen stain (Bensley, 1939) for 10 to 15 minutes. Slides were rinsed thrice in a solution of potassium metabisulphide and were taken through grades of alcohol, cleared in xylol and mounted in canada balsam.

The measurements were taken with the aid of a photovolt photometer, Model 512. The method described by Hellen, Wendler and Hastings (1946) was followed. The image from the microscope was projected at a magnification of 100 times into the search unit of the photometer using 557 μ green filter. A blank was set up using a clean slide and a coverslip. The stained section was interposed and the degree of light absorption as indicated by the photometer was recorded. Four readings were taken in each of the different regions of the sections and the mean was calculated. Readings from the cells of the region of gut and teletroch of the larva were taken, as the concentration of glycogen is almost confined to these regions.

Observations and Results

It was observed that glycogen concentration was relatively greater in the gut and telotroch regions than in the other regions of the larva. Before metamorphosis (stage I) the glycogen content is low but the cells in the telotroch region appear deeply stained. This is supported by the photometric reading showing 15.5 for gut regions and 14.5 for the telotroch region (Table I). About the time of metamorphosis (stage II) the glycogen content increases and the concentration is very high in the gut wall and telotroch. Glycogen accumulates in the form of granules. Optical measurements of the sections in the regions of gut and telotroch show for the gut 43.5 and for telotroch 49.00 (Table II). This clearly indicates the increase of glycogen about the time of metamorphosis. After metamorphosis optical measurements of the sections in the regions of gut and telotroch show for the gut as well as for telotroch 29.5 (Table III). This indicates the decrease of glycogen content after metamorphosis.

Discussion

Cytochemical studies on frog embryos by various authors show that there is considerable decrease of glycogen content during gastrulation. Needham (1950) points out that in chick development, glycogen is present

TABLE I

Photometric measurements of glycogen of *Actinotrocha* larva

Stage I Before metamorphosis 'Blank': 96.

Optical density of the sections					
Gut			Telotroch		
Optical density	Difference from Blank	Mean	Optical density	Difference from Blank	Mean
77	19		78	18	
80	16	15.5	81	15	14.5
84	12		84	12	
81	15		84	12	

TABLE II

Photometric measurements of glycogen of *Actinotrocha* larva

Stage II During metamorphosis 'Blank': 96

Optical density of the sections					
Gut			Telotroch		
Optical density	Difference from Blank	Mean	Optical density	Difference from Blank	Mean
55	41		43	53	
47	49	43.5	48	48	49.0
53	43		51	45	
55	41		46	50	

TABLE III

Photometric measurements of glycogen of *Actinotrocha* larva

Stage III Post-metamorphosis 'Blank': 96

Optical density of the sections

Gut			Telotroch		
Optical density	Difference from Blank	Mean	Optical density	Difference from Blank	Mean
71	25		69	27	
67	29	29.5	66	30	29.5
62	34		61	35	
66	30		71	25	

only in traces at the beginning of incubation and increases steadily afterwards.

In the Japanese beetle (*Popillia japonica*) according to Ludwig and Rothstein (1945) the glycogen content increases from 1.43% in the larva to 2.07% in the early pupa. During the pupal stage there is a rapid loss of glycogen during the first four days, an increase on the fifth day and a decrease throughout the remainder of pupal life. The authors conclude that glycogen provides the energy during the pupal stage.

In the development of *Actinotrocha* larva as shown by the present investigation, the glycogen content which is practically confined to the gut and telotroch regions, is low before metamorphosis and increases three times about the time of metamorphosis, and then decreases to two thirds of this amount in the post-metamorphosis stage. The ratio of glycogen content in the three stages investigated is 1 : 3 : 2. It is very likely that the fall in glycogen is related to the energy requirements of metamorphosis.

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LIPASE ACTIVITY IN THE VERTEBRATE HEART MUSCLE AND ITS RELATION TO BASAL METABOLISM

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MOST of the mechanical energy involved in heart beat is used in expelling blood into the arteries against pressure and the minute volume of circulation or the cardiac output per minute is dependant on the pulse rate and stroke volume (Dukes, 1947). The pulse rate of different animals vary according to size and age. The cardiac output which is calculated by multiplying the stroke volume by the pulse rate is greater per unit weight of the body in small animals. This is indicative of the fact that smaller animals have a higher metabolic rate per unit body weight (Dukes, 1947). Brody (1945) pointed out that as a rule the pulse rate is directly proportional to the basal oxygen consumption per unit body weight in mature animals of different species and is inversely proportional to body size. But Prosser (1950) considers it probable that activity is more important. Variations in heart beat of animals are influenced by the various conditions that affect the basal metabolism of animals in general such as season, age, sex, activity, environmental temperature, pathological conditions etc.

In heart muscle therefore there should be a tremendous expenditure of energy depending on the heart rate. Contrary to the classical belief that carbohydrates form the chief energy source in the heart muscle, the recent work of Bing and his collaborators (Bing, 1954) of sampling the blood drawn from the coronary sinus by the catheter technique—the most reliable method available at present, for studying the metabolism of the heart—have shown that nearly 67% of the required energy in humans is derived from fatty acids and only a portion of the remaining from carbohydrates. It has been observed in birds and in certain other flying animals like bats and locusts that fat is the main fuel during long and sustained muscular activity (George and Jyoti, 1955 and '57, Weis-Fogh, 1952). Our finding that there is a lipase in the vertebrate skeletal muscle (George and Scaria, 1956 and '57, George *et al*, 1957) and insect flight

muscle (George *et al*, unpublished data) and that there is a quantitative variation depending on activity in the different muscles of the same animal and the same muscle of different animals (George and Scaria, 1956 and unpublished data, George *et al*, unpublished data) supports this view. We (1956) also suggested that the lipase concentration of a muscle would depend on the extent of fat utilization which in turn could be correlated with the nature of activity of the muscle. It was therefore thought that the heart muscle which performs sustained activity would show a very high lipase activity and a quantitative difference in different animals depending on the metabolism of the animal. This study was therefore undertaken to see if some experimental evidence could be made available in order to substantiate our assumptions.

Material and Methods

The lipase activity of the heart muscle of the following animals was assessed.

Frog (*Rana tigrina*)	Freshly collected
Turtle (*Lissemys punctata*)	Laboratory animal
Pigeon (*Columba livia*)	Wild
Parakeet (*Psittacula kramari*)	Wild
Kite (*Milvus migrans*)	Wild
Eagle (*Aquila rapax*)	Wild
Sparrow (*Passer domesticus*)	Wild
Fowl (*Gallus domesticus*)	Domestic
Bat (*Rhinolophus* sp.)	Wild
Rat (*Rattus rattus*)	Wild
Rabbit (*Oryctolagus cuniculus*)	Laboratory animal
Sheep (*Ovis aries*)	Slaughter house material, collected immediately after decapitation
Horse (*Equus caballus*)	Heart cut out about an hour after death

Enzyme Material

A glycerine extract of the heart muscle was prepared as described in our earlier paper (George and Scaria, 1956). In the case of small animals like sparrow, rat, bat and parakeet a number of them irrespective of age and sex were sacrificed for each set of experiments to procure the required quantity of the muscle. The blood was completely removed with filter paper and only the muscle of the ventricle used for the assay.

Method

The method of estimation was essentially the same as used in our previous study, (George and Scaria, 1956) but in many cases the quantity of the reaction mixture was reduced to one half and contained 5ml. of the emulsion, 2.5 ml. buffer and 2.5 ml. enzyme preparation, thus making up a total volume of 10 ml. and correspondingly the quantity of the alcohol—acetone mixture used to stop the reaction was also reduced to one half. The pH was adjusted to 8 and the reaction mixture was incubated for 4 hours at 40°C.

Calculation

The lipase activity is referred to as the lipase value which is expressed as the number of lipase units per gm. of the wet muscle and was calculated according to the definition of George and Scaria (1956).

Results

The values obtained for the heart of the different animals is given against the heart rate and/or the metabolic rate in the respective animals taken from available previous literature. (Table 1)

TABLE 1

Data on the relation between the metabolic rate and the lipase value of the heart muscle of various animals

Animal	Heart rate (Author in parentheses)	Metabolic rate		Lipase value
		Oxy. conc. cc./ gm./hr. (Author-Heilbrunn)	Heat prod. 24 hrs./ kg. body weight (Author-Brody)	
Frog		0.21		6.4
Turtle				3.3
Pigeon	185 (Heilbrunn)		102	11.2
Parakeet	320 (Heilbrunn)		227	10.6
Kite				11.1
Eagle				10.4
Sparrow	800 (Heilbrunn)	6.71	231	26.7
Fowl	150-180 (Prosser)	0.83	52	9.4
Bat				15.2
Rat	300-500 (Prosser)	0.692	83	22.2
Rabbit		0.64	45	27.7
Sheep	70-80 (Dukes)	0.34	26	6.7
Horse	32-44 (Dukes)	0.25	17	3.9

Lipase Value in Relation to Age and Sex

The animals chosen for this study were rats and sparrows, the sparrows for sex differences and the rats for sex as well as age. The weight of the animals were determined and used as an index of age. Thus the rats were classified into the following three weight groups.

A Above 100 gm.
B 50-100 gm.
C Below 50 gm.

In the first two groups the males and females were separated and the lipase value of the heart assessed separately. A number of animals were sacrificed for each experiment and the values given below are the average of three experiments each one done in duplicate.

TABLE 2

Showing the lipase value of the heart muscle of rat and sparrow in relation to age and sex

Animal	Group	Lipase value	
		Male	Female
Sparrow		24.0	22.0
Rat	A	26.4	17.6
,,	B	28.2	23.0
,,	C	18.5	

Discussion

According to Kleiber (1932) the influence of body size on metabolism may reasonably be related to oxygen transport. Henderson's (1923) results demonstrated a direct proportionality between circulation rate and pulse rate and it has been suggested that pulse rate be used for determining metabolic rate. So if the size of the heart were directly and the pulse rate inversely proportional to the body weight, in small animals, per unit weight of the body, the heart muscle should expend a greater amount of energy than in larger animals. It has been shown (Green, 1954) that the heart muscle is equipped with all the enzymes necessary for the oxidation of fatty acids. If fat is the major source of energy for the activity of the heart, the heart muscle of smaller animals should have a better equipment of the enzyme systems responsible for the degradation

of fat into the final products of oxidation. The concentration of lipase in the heart muscle therefore is important because this is the enzyme concerned with the primary step in fat utilization namely the breakdown of fat into fatty acids and glycerol. Table 1 shows that there is a difference in the lipase value of the heart in different animals and it roughly conforms to the difference in the metabolic rate. A similar difference may also be expected in the case of the other enzymes of the "fatty acid cycle". Since the values presented in Table 1 cannot be considered as absolute, it is not our intention to formulate any mathematical relationship between metabolism and lipase value. It only shows that there is a significant relationship between the lipase value and the metabolic rate and supports the assumption that mostly fat is utilized for energy purposes in the heart and our own contention that the lipase concentration of a muscle would depend on its activity and consequently the utilization of fat in the muscle.

Table 2 shows the lipase activity of the heart muscle of rat in relation to age and sex and of sparrow in relation to sex. In both cases there is an appreciable difference in the lipase value of the male and female, the male having a higher value and it is known that the metabolic rate of the male is higher than that of the female. Among the three age groups in rats, the highest value is obtained for the B group. This is in conformity with the age curves of metabolism in rats. According to the figures of Brody (1945) the metabolism per unit surface area rises and in the case of rats it rises from about 400 cal/sqm/day near birth to 1100-1200 at the age of 40 days or body weight of 100 gm. Thereafter the metabolism declines to about 800 cal/sqm/day at the age of few months or weight of 300 gm. It might therefore be concluded that the mechanism behind these metabolic changes is enzymic and shows the ability of the animal to derive energy by metabolizing high energy metabolites by the development of the appropriate enzyme systems.

Summary

1. The lipase value of the heart muscle of a number of vertebrates is determined.

2. It is found that the heart lipase value in different animals, vary according to size, sex, age and activity. As a rule smaller animals have a higher lipase value than larger ones. In rat and sparrow the males have lipase value higher than females. In rat, lipase activity of the heart is

highest during the period of maximum growth. These changes in the lipase activity of the heart muscle is correlated with metabolic rate.

3. The significance of the high concentration of lipase in the heart muscle is discussed.

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A STUDY OF THE URINARY NITROGEN EXCRETION LEVEL IN NORMALS AND ITS RELATION TO DIETARY PROTEIN INTAKE

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As early as in the year 1908, McCay studied forty-four subjects including Bengali students, watchmen, domestic servants etc. and made observations on low levels of urinary nitrogen in all of them, excepting one subject. The average urine nitrogen gave a value of 5.98 gm. per day. He assumed that urine nitrogen indicated the level of protein metabolism in the body and that in the case of normal healthy adults who were able to maintain a constant body weight, it gave an idea of the protein intake. This led him to conclude from his above studies that the subjects lived on a diet containing low protein. These observations were later followed by similar types of studies by workers from different parts of India. Ray and Ganguly (1938) studied fifty subjects and found that the total urinary nitrogen excretion gave an average value of 4.83 gm. per day. Gokhale (1941) in Bombay observed in 47 male healthy adults an average of 6.09 gm. of urinary nitrogen excretion per day, with a range of 3.62 to 9.84 gm. Thus the average urinary nitrogen excretion of Indian adults as found by various workers has been taken to be in the neighbourhood of 5—6 gm. per day. Patwardhan *et al* (1949) and Karambelkar *et al* (1950) obtained some interesting results on nitrogen metabolism, in Indian adults. The authors stated that in the predominantly vegetarian composition of Indian dietaries probably lies the secret of the low urine nitrogen observed in Indians and not in the low intake of protein or low digestibility of the protein contained in these diets (Patwardhan, 1952).

Comparatively little data is available on the studies of urinary nitrogen excretion levels in Indian adults living in this part of the country, namely Gujarat. As it is true that the daily excretion of nitrogen is an index of the nutritional level of the individual in regard to protein, it was thought worthwhile to take up the study of determining the daily nitrogen excretion in urine of male adults belonging to this region of Gujarat and to make an attempt to correlate these findings with their dietary protein levels.

Material and Method

Eighteen normal male healthy subjects belonging to ages varying between 17 and 22 years, were studied for their daily out-put of nitrogen in urine. They were all vegetarians. Their average daily diet included rice, chapati, tuver-dahl prepared in the liquid form *i.e.* the form as is customary here on this side, and vegetable, mostly the type of the leafy vegetable like radish, 'tandulja' or sometimes 'dudhi', cabbage, cauliflower etc. which are particularly in abundance here in the different seasons of the year. Milk and/or preparations of milk, like curds and butter-milk, are included in their dietaries. Very few of these subjects, however, had no objection to taking eggs, but none of the above subjects took eggs and/or egg preparations except perhaps on very rare occasions. The data regarding their average daily dietary intake was collected and the average nutritional level of the individual in regard to the protein intake has been calculated. This has been shown in the table (vide table I). A representative sample from the 24 hrs. collection of urine collected under toulene as preservative was used for analysis after ensuring by preliminary tests that each sample was free from protein and sugar (Hawk *et al*, 1947). The results of the analyses are shown in the Table I.

TABLE I

Daily Urinary Nitrogen Excretion in Normals

Sr. No.	Subject	Age in years	Nature of diet taken	Average total protein intake in diet gms/day	Vol. in c. c. of 24 hours urine	Total urinary nitrogen in gms./day
1	P. P.	20	Vegetarian	61.6	1282.0	5.74
2	C. P.	17	Vegetarian	53.0	655.0	4.87
3	D. P.	18	Vegetarian	76.1	1450.0	8.10
4	N. M.	21	Vegetarian	45.6	804.0	4.85
5	T. C.	20	Vegetarian	46.8	452.0	5.97
6	P. F.	20	Vegetarian	53.4	805.0	5.30
7	J. V.	19	Vegetarian	69.1	950.0	7.10
8	K. B.	20	Vegetarian	68.6	2409.0	7.39
9	B. R.	18	Mixed	54.5	1362.0	5.33
10	J. A.	17	Vegetarian	52.5	990.0	6.40
11	P. S.	20	Vegetarian	45.5	1540.0	6.30
12	B. D.	18	Mixed	72.0	539.0	5.96
13	S. C.	22	Vegetarian	65.0	672.0	4.82
14	D. T.	19	Mixed	56.0	820.0	5.90
15	P. R.	22	Vegetarian	69.0	1140.0	7.66
16	V. A.	20	Vegetarian	63.0	1370.0	5.60
17	P. A.	19	Vegetarian	68.0	852.0	5.70
18	S. J.	22	Vegetarian	43.8	560.0	3.20

TABLE II

Average daily dietary protein intake etc. in normals

	Average total protein intake in diet gms./day	Vol. in c.c. of 24 hrs. urine	Total urinary nitrogen in gms./day
Maximum	76.1	2409	8.10
Minimum	43.8	539	3.20
Mean	59.1	1036	5.90

TABLE III

Comparison of nitrogen excretion values obtained by different workers

	McCay (1908) In Bengal	Ray and Ganguly (1938) In Bengal	Gokhale (1941) In Bombay	Narayanan (1935) In South India	Present series In Gujarat
Average total urinary nitrogen in gms./day	5.98	4.83	6.09	7.1	5.90

Results and Discussion

In Table. I, the results of daily urinary nitrogen excretion, the volume of 24 hours' urine out-put and the average daily intake of proteins in the diet of the normal subjects studied have been shown. As can be seen from tables I and II, the total nitrogen excreted in urine per diem by these subjects varied from 3.20 to 8.10 gm. with the mean value of 5.90 gm. In table III, the daily urinary nitrogen excretion values obtained by different workers, in their subjects belonging to different parts of India have been compared. It will be revealed from this table of comparison that the values obtained in the present series are very well comparable with those of McCay (loc-cit) and of Gokhale (loc-cit). The present values are slightly higher than those of Ray *et al* (loc-cit) whereas they are slightly lower than those of Narayanan (1935). Narayanan (loc-lit) obtained in South Indian subjects a higher range of 4.1 to 11.1 gms. per day with the average value of 7.1 gms. per day for the daily total urinary nitrogen excretion. All these values are, however, definitely lower than the corresponding standard value given for Europeans, which is 16 gm. per day.

Regarding the average total protein intake in diet of these subjects, the calculated value has been found to be ranging between 43.8 to 76.1 gm. per day with the average value of 59.1 gm. per day (vide tables). In the dietary studies of Aykroyd and Krishnan (1937) who investigated the diets of various villagers in South India, it was found that the average protein intake per head was about 50 gm. The value obtained in the present series is thus very well comparoble with that given by the above authors. The minimum protein requirement of the diet is a matter on which there is no agreed scientific opinion. Many authorities like the nutrition committees appointed by British Medical Association (1933) have recommended that 100 gm. protein are sufficient to maintain the health and activity of an average man. Health committee of the league of Nations (1935) recommended that the protein intake for adults should not fall below 1 gm. per Kg. body weight. According to these standards thus the protein intake obtained in the present series is lower than what is required. Secondly as can be seen from the Table the diet taken by the subjects in the present series is, in the majority, a vegetarian type, in contrast to the animal protein rich diet of the non-vegetarian type.

The above findings once more bring out the fact that the standards of excretin of urinary nitrogen based on the studies on Europeon subjects cannot be accepted for Indians, for whom lower standards, which will have to be obtained from the collected data of studies carried out in different regions of country, will have to be made applicable.

Summary and Conclusions

(1) Eighteen normal male healthy subjects of ages varying between 17 and 22 years living on this side of the country namely, Gujarat, have been studied for their daily urinary nitrogen excretion.

(2) The urinary nitrogen excretion ranged from 3.20 to 8.10 gm. per day, with the average value of 5.90 gm. per day. This is much lower compared to the corrosponding standard value given for Europeans, which is 16 gm. per day.

(3) The average total protein content of the daily diet taken by the subjects was 59.1 gm. with a range of 43.8 to 76.1 gm. This protein content also seems to be lower than the standard value recommended by league of Nations' Health Committee etc.

(4) The diet of the majority of the subjects was mostly of vegetarian type, low in animal proteins as compared to the animal protein rich

non-vegetarian type. This factor, namely the vegetarian composition of diet, may perhaps be responsible for the low urinary nitrogen excretion in them.

(5) These findings once more bring out the fact that theEuropean standards of excretion of urinary nitrogen, cannot be accepted for Indians, for whom, lower standards, obtained after studies made on subjects belonging to different regions of the country, will have to be made applicable.

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STUDIES ON THE STRUCTURE AND PHYSIOLOGY OF THE FLIGHT MUSCLES OF BIRDS

2. The Relative Reduction of Fat and Glycogen in the *Pectoralis Major* Muscle during Sustained Activity

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IN an earlier paper we (1955) published data on the reduction of fat in the *pectoralis major* muscle and liver of some birds and a bat during exercise effected by direct electrical stimulation of the muscle. Of the various birds studied, the fowl, kite and pigeon representing the nonflying, soaring and flapping types respectively, are chosen for the present study. The figures for the reduction of fat in the muscle and liver reported earlier and also those for the reduction in the glycogen content of these parts, also obtained in the same experiments are now presented. In addition experiments were conducted on the pigeon subjecting it to forced flight and also electrical stimulation after forced flight and the respective values for the reduction of fat and glycogen in the muscle and liver obtained in these sets of experiments with a view to determining which of the two energy fuels contributed the major part of the energy required for such sustained muscular exercise.

Material and Methods

The values for glycogen given in table 1 were obtained from the same animals as were used for the estimation of fat (George and Jyoti, 1955). A piece each of the muscle and the liver was cut out before and a piece of the muscle on the other side after stimulation and the glycogen and fat estimated separately in the same individual. The animal chosen for forced flight and stimulation was the pigeon. A pigeon was made to fly in a large hall till it was fatigued and was immediately pithed. The liver and muscle samples were cut for the estimation before and after stimulation as above.

The total lipid content of the muscle and liver was estimated separately as per the methods employed in our previous study. The method used for the estimation of glycogen was a colorimetric micro-

method according to Kemp *et al* (1954) using the Beckman's Spectrophotometer (DU Model).

Results

The results obtained are given in the tables below. In giving the fat and glycogen contents however the range in variation as well as the mean values are given because there is considerable individual variation depending upon the physiological state of the animals. It is assumed that the reduction in the fat and glycogen contents of the breast muscle and liver noted at the end of the continuous muscular exercise denotes the amount of the substances utilized for energy. Reduction if any in the protein was very slight and it was not taken into account for the present study. The respective energy values were obtained by multiplying the fat reduction figure by 9.5 and the glycogen reduction figure by 4.2 (Krogh and Lindhard, 1920).

TABLE I

Glycogen content and its reduction in the *pectoralis major* muscle and liver after electrical stimulation *

	Pigeon				Kite				Fowl			
	Muscle		Liver		Muscle		Liver		Muscle		Liver	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
Percentage before stimulation	0.31	0.28-0.35	3.03	2.72-3.30	0.73	0.69-0.77	2.55	2.00-3.00	0.54	0.50-0.57	2.39	2.12-2.67
Percentage after stimulation	0.28	0.26-0.30	2.05	1.78-2.29	0.37	0.35-0.40	1.76	1.58-1.90	0.48	0.45-0.50	1.71	1.66-1.77
Actual reduction	0.03	0.02-0.05	0.98	0.94-1.01	0.36	0.34-0.37	0.79	0.42-1.10	0.06	0.05-0.07	0.68	0.46-0.90

* The values given are the mean of five experiments.

TABLE 2

Actual reduction in the total lipid and glycogen contents of the *pectoralis major* muscle and liver after electrical stimulation of the muscle with the respective and total energy values.*

	Pigeon	Kite	Fowl
Total reduction of lipid per 100 gm. each of muscle and liver	1.10 gm.	0.78 gm.	0.52 gm.
Total energy value for lipid reduced in 100 gm. each of muscle and liver (L)	10.45 Cal.	7.41 Cal.	4.94 Cal.
Total reduction of glycogen per 100 gm. each of muscle and liver	1.01 gm.	1.15 gm.	0.74 gm.
Total energy value for glycogen reduced in 100 gm. each of muscle and liver (G)	4.25 Cal.	4.73 Cal.	3.11 Cal.
Total energy value for lipid and glycogen reduced in 100 gm. each of muscle and liver (L + G)	14.70 Cal.	12.14 Cal.	8.05 Cal.
Percentage of total energy obtained from lipid alone $\frac{L \times 100}{L + G}$	71.08	61.02	61.37
Percentage of total energy obtained from glycogen alone $\frac{G \times 100}{L + G}$	28.92	38.96	38.64

* The values for fat were taken from our earlier paper (1955).

TABLE 3

Total lipid and glycogen contents of the *pectoralis major* muscle and liver and their reduction after (1) forced flight (2) forced flight and electrical stimulation in the pigeon*

	Lipid		Glycogen	
	Muscle	Liver	Muscle	Liver
Percentage before flying	4.46	3.88	0.31	3.03
Percentage after flying	2.99	2.28	0.18	1.75
Actual reduction after flying	1.47	0.60	0.13	1.28
Percentage after flying and stimulation	2.87	3.24	0.17	1.72
Actual reduction after flying and stimulation	1.59	0.64	0.14	1.31

* The values given are the average of five experiments. The figures for fat and glycogen before flying given were obtained from the previous experiments and are considered to represent the respective normal average values.

TABLE 4

Actual reduction in the total lipid and glycogen contents of the *pectoralis major* muscle and liver with the respective and total energy values calculated from figures given in table 3

	After flying		After flying and stimulation	
	Lipid	Glycogen	Lipid	Glycogen
Total reduction per 100 gm. each of muscle and liver	2.07	1.41	2.23	1.44
Total energy value in Calories for the quantity reduced in 100 gm. each of muscle and liver	19.66	5.94	21.18	6.09
Percentage of total energy obtained	76.81	23.20	77.64	22.39

Discussion

The pigeon breast muscle (*pectoralis major*) contains about the same amount of fat as that of the kite and about five times that of the fowl. In respect to glycogen the kite breast muscle contains the highest amount which is more than double that of the pigeon. It is observed that after electrical stimulation of the breast muscle till it was fatigued, the reduction in the lipid and glycogen contents per 100 gm. of the muscle as well as 100 gm. of liver in the case of the pigeon, kite and fowl corresponds to a total energy value of about 15, 12 and 8 Calories respectively (table 2) calculated from the sum total of the amounts of fat and glycogen reduced in both muscle and liver together. Of these energy values again, in the pigeon, kite and fowl 71%, 61% and 61% respectively (table 2) of the energy utilized was obtained from lipid alone while the remaining only (table 2) from glycogen.

In the second set of experiments (table 3) in which a pigeon was subjected to forced flight continuously till it was fatigued about 77% (table 4) of the total energy expended was obtained from fat only while the remaining only from glycogen. In the third set of experiments in which the pigeon was subjected to forced flight till fatigued and then the muscle electrically stimulated, it was found that about 78% of the total energy expended was obtained from fat and the rest from glycogen.

The general conclusion that can be drawn from this study is that stored fuel in the flight muscles of birds is to a great extent fat and that this material is the chief fuel in these animals. Weis-Fogh (1952) who

studied locusts concluded that the long range migration of small flying animals like locusts could only be possible if fat were to be used as fuel. In the light of these findings it is possible to explain how birds like the golden plover obtain the necessary energy to fly long distances at a stretch without food nor rest during migration. Prior to migration large amounts of fat are stored in the form of muscle and depot fat, and the depot fat should be made available to the muscle when the muscle fat gets depleted. This aspect is under investigation in our laboratories.

Summary

1. Three birds—the pigeon, kite and fowl were exercised and the reduction of fat and glycogen in the *pectoralis major* muscle and liver estimated to find out as to what is the chief fuel for such activity in these animals.
2. It was found that when the pectoral muscle was electrically stimulated, of the total energy expended 71% in the case of the pigeon and 61% in the kite and fowl were derived from fat.
3. When the pigeon was subjected to forced flight till it was fatigued, 77% of the total energy expended could be accounted for, from fat.
4. In a pigeon which was fatigued after forced flight and was further exercised by electrical stimulation it was found that about 78% of the energy came from fat and only the rest from glycogen.

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THE pH AND TITRATABLE ACIDITIES OF FRUITS AND THE EFFECT ON KEEPING

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THe nutritional importance of fruits in dietetics and their influence in body metabolism have been well recognized since long. One of the important functions of fruits, which is related to its mineral content, is its effect upon the acid-base balance of the body. Fruit is relatively low in acid elements namely phosphorus, sulphur and chlorine, and higher in basic elements e. g. sodium, potassium and magnesium. In metabolism an alkaline ash formed will help to neutralize the acid ash of cereal foods and meat. The citric acid compounds of lemon, grapes and oranges, and the malic acid of apples, peaches, berries etc. are easily burnt in the body to leave an alkaline residue. The acids in prunnes, plums, cranberries and rhubarb are not well utilized in the body and for this reason these fruits should be classed as potentially acid, although they do have an alkaline ash.

The digestibility of fruit in the stomach and intestine is dependent largely on the nature of the fruit and its degree of ripeness. On the other hand if the fruit is unripe and the amount of cellulose consequently greater, digestion may be much more prolonged. The excess of acids present in unripe fruits causes it to be irritating to the intestine and frequently promote diarrhoea and colic disorders. If, however, the cellulose and acids are present in more moderate quantiteis in ripe fruit, the gentle stimulation which they exert on the intestinal wall may be very useful. During digestion, fruits retain their natural acids, which are an aid to the passage of food, except when an oversupply of hydrochloric acid is present in the stomach.

Another aspect of the role played by fruits in body metabolism that has been studied is with regard to their enamel erosive properties. A number of investigators (McClure 1943; Gortner, Restarski, Bieri & McGay, 1945; Bieri, McCay, Restarski, & Gorter, 1946; and Wynn and Haldi, 1948) showed that acid beverages, natural or synthetic, when fed to rats caused destruction of the lingual enamel of ther molar teeth.

Miller (1950) in his study on enamel erosive properties of fruits and fruit juices, determined their pH and the titratable acidities. He found that the difference in their effect on enamel could not be related to their difference in their titratable acidity.

A study was undertaken here in this laboratory to find out the relationship between the pH and the titratable acidities of some fruits which are commonly eaten by the people in this area, and also to study the effect of keeping on their properties at the temperature of the refrigerator on the one hand and at room-temperature on the other.

Material and Method

Ripe fruits of good quality were obtained in fresh condition. Care was taken to see that fruits of one variety, which were to be studied for their effect of keeping them at different temperatures on the pH and the acidity of their extract, belonged to the same picking, so that they could be divided in three equal lots, which were identical in every other respect for comparative study. The fruits were weighed before removing the skin, wherever it was found necessary to peel of the skin in order to separate it from the edible portions and the weight of the edible part was found out after deducting the weight of the skin as also of the seeds which were recovered and weighed, in such of those cases where they were to be removed from the edible portion. A known amount of glass double distilled water was added for extracting the juice from the pulp which was first sliced in order to have a homogeneous extract. (This made it possible to calculate the amount of extract equivalent to the edible portion). The extract was then filtered through chemically pure grade filter papers, after having taken proper precautions for preventing as far as possible its contamination with atmospheric carbon dioxide, all throughout the experiment. Under these identical conditions the other two lots, one kept at the temperature of the refrigerator and the other at room-temperature for a certain period of time varying between one to five days, were also treated for preparing the extract. These samples of the extract from the fruits thus obtained were divided into two portions. The pH was determined by means of the Beckman pH meter, G model and the titratable acidity determination was done after titrating against N/10 NaOH to pH 8.1 by use of the indicator (A. O. A. C. 1950). The pH meter was standardized first against the standard buffer of pH 7 before taking the reading for pH which was taken in duplicate and the average values calculated.

Results

In the table given below the results for the pH values obtained and those for the titratable acidity determination are shown. In case of the titratable acidity readings, which were also obtained after standardization and in duplicate, they are expressed as equivalent to cc. of N/10 sodium hydroxide required for neutralization of 1 cc. of the extract. This method of expressing acidity in terms of cc. of N/10 NaOH required for neutralization by titrating it to pH 8.1 is mostly followed after that of Miller (1950), with slight modification in that the readings are expressed here as per cc. of the extract instead of the total bulk of the fruit as it is found to be suitable for comparative evaluation of the results of the determinations, which are made under identical conditions of extractions etc. as mentioned above.

Table showing the pH and titratable acidity of fruits

Sr. No.	Fruits: Local Name	Botanical Name	Particulars	Extract ≙ gms. of fruit (pulp)	pH.	Titratable acidity in cc. of N/10 acid per cc. of Extract
1	2	3	4	5	6	7
1	Chiku	Achras Sapota	Fresh	161.9	5.70	0.29
			* for 2 days	128.5	5.80	0.02
			for 5 days	126.0	4.85	0.14
			† for 2 days	45.1	5.65	0.03
			for 5 days	45.1	5.45	0.05
2	Jamrukh	Psidium Guyava	Fresh	126.4	4.50	0.33
			* for 4 days	125.0	4.35	0.41
			† for 4 days	122.0	4.15	0.28
3	Mosambi	Citrus aurantium var. dulcis	Fresh	108.3	4.70	0.22
			* for 5 days	105.0	4.70	0.30
			† for 5 days	103.0	4.65	0.33
4	Narangi	Citrus nobilis var. deliciosa syn. citrus chrysocarpa	Fresh	68.1	3.90	0.77
			* for 2 days	67.0	3.92	0.88
			† for 2 days	68.0	3.85	0.83

* Kept at room temperature, this varied between 15° to 25°c during the period of the experiments.

† Kept at the temperature of the refrigerator.

1	2	3	4	5	6	7
5	Safarchand	Pyrus malus	Fresh	105.6	3.90	0.35
			* for 4 days	101.0	3.72	0.48
			† for 4 days	102.5	3.87	0.32
6	Darak	Vitis vinifera	Fresh	65.5	3.10	1.55
			* for 2 days	64.0	2.92	2.15
			† for 2 days	63.0	3.05	1.40
7	Dalam	Punica Granatum	Fresh	137.0	3.50	0.40
			* for 4 days	132.0	3.20	0.54
			† for 4 days	135.0	3.30	0.46
8	Amla	Phyllanthus emblica	Fresh	84.0	2.80	2.70
			* for 4 days	81.0	2.70	3.36
			† for 4 days	83.0	2.70	2.40
9	Rajberry	Rubus idaeus	Fresh	43.5	3.70	2.15
			* for 4 days	41.0	3.80	0.90
			† for 4 days	42.0	3.62	1.10
10	Bor	Zizyphus jujuba	Fresh	43.0	3.30	0.74
			* for 4 days	40.0	3.30	0.70
			† for 4 days	41.0	3.35	0.55
11	Tomato	Lycopersicum esculentum	Fresh	67.0	4.12	0.70
			* for 5 days	64.0	4.42	0.42
			† for 5 days	65 0	4.20	0.38
12	Khasadia-kela	Musa paradisiaca var. sapienum	Fresh	124.0	4.72	0.30
			* for 4 days	120.0	5.35	0.28
			† for 4 days	122.0	4.82	0.30
13	Lalkela	Musa paradisiaca	Fresh	70.0	4.50	0.40
			* for 5 days	65.0	4.75	0.35
			† for 5 days	66.0	4.25	0.66
14	Kashibor	Zizyphus xylopyrus	Fresh	60.0	4.65	0.15
			* for 1 day	59.0	4.45	0.17
			† for 1 day	60.0	4.25	0.15
15	Gorasamli	Pithecolobium-dulcis	Fresh	26.0	4.95	0.30
			* for 1 day	25.0	4.87	0.37
			† for 1 day	26.0	4.87	0.27
16	Totapurikeri	Mangifera indica	Fresh	103.0	5.10	0.05
			* for 4 days	103.0	4.10	0.15
			† for 4 days	103.0	4.65	0.06
17	Hapuskeri	Mangifera indica	Fresh	52.0	4.05	0.14
			* for 1 day	52.0	4.90	0.15
			† for 1 day	52.0	4.75	0.13

* Kept at room temperature, this varied between 15° to 25°c during the period of the experiments.

† Kept at the temperature of the refrigerator.

1	2	3	4	5	6	7
18	Lokat	Eriobotrya japonica	Fresh	19.0	3.20	0.76
			* for 1 day	18.0	3.30	0.74
			† for 1 day	18.0	3.27	0.69
19	Rayan	Mimusops elengi	Fresh	50.0	5.40	0.06
			* for 1 day	48.0	5.38	0.05
			† for 1 day	47.0	5.32	0.07
20	Ramphal	Anona-reticulata	Fresh	27.0	4.75	0.26
			* for 1 day	27.0	4.93	0.10
			† for 1 day	27.0	5.00	0.19
21	Kachakeri	Mangifera indica	Fresh	60.0	2.70	1.80
			* for 4 days	58.0	2.84	1.75
			† for 4 days	55.0	2.75	2.00
22	Papayi	Carica papyya	Fresh	41.0	4.87	0.10
			* for 2 days	41.0	4.25	0.23
			† for 2 days	41.0	5.45	0.10
23	Tarbuj	Citrullus vulgari	Fresh	52.0	5.03	0.06
			* for 2 days	52.0	5.02	0.07
			† for 2 days	52.0	5.05	0.09
24	Teti	Cucumis melo	Fresh	61.0	6.03	0.06
			* for 2 days	61.0	6.02	0.07
			† for 2 days	61.0	6.40	0.05
25	Badam (dried)	Prumus amygdalus	—	38.0	6.20	0.12
26	Darak (dried)	Vitis vinifera	—	35.0	3.72	0.60

Discussion

In the table, the weight of the fruits given is that of the pulp *i e.* the edible portion of the fruit without the skin and the seeds, wherever such fruits possess these latter portions and it is a usual practice to do so amongst the people, before they eat these fruits. Otherwise in the remaining cases the given weight was that of the whole fruit as such with the skin and/or the seeds. The period of keeping these fruits either at the room temperature or at the temperature of the refrigerator which was, however, same for both, varied between one to five days, depending on the condition that the fruit remained in a good state for human consump-

* Kept at room temperature, this varied between 15° to 25°c during the perid of the experiments.

† Kept at the temperature of the refrigerator.

tion during that interval. The fruits obtained from the same picking were selected in such a way that they were more or less equal in size and in other qualities as well so that as can be seen from the figures of their weights in the table they could be as far as possible equally divided into three lots for analysis under three conditions namely, fresh, after keeping at room temperature and at the temperature of the refrigerator. This procedure, as has already been explained above, under methods, gave identical portions for comparison of the pH and the titratable acidity determinations of their extracts. An attempt was made as in case of item No. 1 of the table to find out the difference if any of significance, in the pH and titratable acidity on keeping the fruits for varying periods at the room temperature and that of the refrigerator. Later on, however, the period was fixed at one level only according to the condition that the fruit remained in good order and did not deteriorate during that interval as mentioned above.

It will be seen from the table that there is no significant difference in the pH or in the titratable acidity of the fruits when they were either analysed in their fresh condition or after keeping them at the room temperature or the temperature of the refrigerator, though in many cases a trend was found to be towards the increasing of the titratable acidity on keeping the fruits at the room temperature than when analysed for their titratable acidity either in fresh condition or after having kept them at the temperature of the refrigerator. There was no relationship, however, to be found between the pH of the fruits on one hand and their titratable acidity on the other hand. Thus it may be seen from the table that with identical pH values, varying values of titratable acidity were obtained and vice versa. This may possibly be due to the fact that the amounts of the weak acids which were present might be varying in different fruits and thus were responsible for their variable titratable acidity readings but they did not contribute much towards the hydrogen in concentration, with the result that not much variation in their pH values was observed. The pH of the fruits studied has been found to vary between 2.70 to 6.40. But in the majority of them it varied between 3.0 to 5.0, though the above were the extreme ranges obtained. Goldmann (1949) in her studies on the pH of fruit juices had reported a pH range of 2 to 5 but had stated that it was chiefly lying between 3 to 4. There was a much wider range observed in the present series for the titratable acidity readings which fell between 0.02 to 3.36 cc. of N/10 acid per cc. of extract.

Summary

(1) The pH and titratable acidity were determined for fruits of different kinds in their fresh condition and after keeping them at the room temperature and that of the refrigerator.

(2) pH of these fruits was found to vary between 2.70 to 6.40; but in majority of them the range was between 3.0 and 5.0.

(3) The titratable acidity readings varied between 0.02 to 3.36 cc of N/10 acid per cc. of extract.

(4) There was no relationship between the pH of the fruits on one hand and their titratable acidity on the other.

(5) These results have been compared with those of other workers and the significance of these findings has been discussed.

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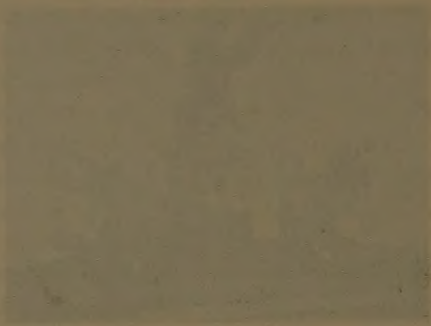

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